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THE GENETICS OF PATHOGENIC ORGANISMS

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E. C. Stakman, Chairman
R. E. Coker
L. O. Kunkel
G. B. Reed
Malcolm H. Soule
E. A. Watson

Edited by
Forest Ray Moulton

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FOREWORD

The Genetics of Pathogenic Organisms is the third symposium in the field of the biological sciences that has been published by the American Association for the Advancement of Science. The first was *The Migration and Conservation of Salmon* (1939) and the second was *Problems of Lake Biology* (1939). A fourth, *The Cell and Protoplasm,* will be published in the near future. Five symposia have been published in the field of medicine and public health—*The Cancer Problem* (1937), *Tuberculosis and Leprosy* (1938), *Syphilis* (1938), *Mental Health* (1939) and *The Gonococcus and Gonococcal Infection* (1939), and two have been published in the field of chemistry—*Applications of Surface Chemistry in Biology* (1938) and *Recent Advances in Chemical Physics* (1938).

This symposium, as in the case of earlier symposia, was planned by experts in its field and all contributions to it were invited. Its inception was due to Drs. E. C. Stakman, R. E. Coker and E. R. Long, who had been considering the subject in a committee of the National Research Council, of which Dr. Stakman was chairman. Now in its completed form the symposium constitutes a comprehensive, authoritative and documented exposition of what has been learned and what needs to be further investigated in an important and relatively new field of science.

If the proper study of mankind is man, the proper study of all higher plants and animals would be the various species themselves. But recently it has become clear that many of the most fundamental problems of biology, even those pertaining to higher plants and animals, can be most effectively attacked on the plane of the lower organisms. Certainly no problem is more important than that of genetics. Whether a human or a protozoan, a tree or a bacterium, it is believed to be its genes that determine its characteristics and the nature of its offspring. Moreover, the law of transmission of genes from one generation to the next is probably essentially the same in all forms, except for differences due to two parents instead of one.

It is therefore advantageous to investigate the general subject of genetics in low forms in which a thousand successive generations can be followed instead of only a few. Fortunately some of these organisms descend from two parents and others from one. Moreover, some simple living units, such as certain kinds of viruses and bacteria and rusts of wheat, consist of large numbers of strains, from one to another of which it seems probable that mutations are most likely to occur and be observed. Although in many cases different strains of an organism are morphologically indistinguishable from one another and not separable by chemical means, such as staining, their pathogenicity to their hosts are often so amazingly specific and marked that they can easily be identified and followed. It is for this reason that *pathogenic* organisms are much more advantageous in genetical investigations than are those which live upon their hosts without producing serious effects. And the specificity of microorganism to hosts emphasizes the importance of the whole question of environment, the effects of which are a part of the problem of variation.

Perhaps in the practical results obtained in breeding farm crops that are resistant to viruses and rusts and other pathogenic organisms, scientists have prepared the way for investigations that in the long run will be even more important than protecting our food, for in this work they have opened new approaches to the great problem of inheritance and evolution. Man is naturally more interested in his own species than in any other and looks anxiously to its future. It now appears that he is most likely to learn the essentials of his own physical being, including the principles by which he may eventually direct his own evolution, from the study of low pathogenic forms of life of which he was until fairly recently quite ignorant and the existence of which he even yet generally regards as a misfortune without one compensating advantage.

F. R. Moulton

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LIST OF CONTRIBUTORS

J. J. Christensen, M.S., Ph.D.
Professor of Plant Pathology and Associate Plant Pathologist, Division of Plant Pathology and Botany, University of Minnesota, University Farm, St. Paul, Minn.

J. H. Craigie, Ph.D.
Pathologist in Charge, Dominion Rust Research Laboratory, Winnipeg, Man., Canada

Clay G. Huff, Sc.D.
Associate Professor of Parasitology and Secretary of the Department of Bacteriology and Parasitology, The University of Chicago, Chicago, Ill.

H. Konst, V.S., D.V.M.
Pathologist, Animal Diseases Research Institute, Department of Agriculture, Hull, Que., Canada

L. O. Kunkel, Ph.D.
Member, The Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, N. J.

G. B. Reed, Ph.D.
Professor of Bacteriology, Queen's University, Kingston, Ont., Canada

A. J. Riker, M.A., Ph.D.
Professor of Plant Pathology, University of Wisconsin, Madison, Wis.

H. A. Rodenhiser, Ph.D.
Plant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

E. C. Stakman, M.A., Ph.D., Dr. Nat. Sc. (Hon.)
Professor of Plant Pathology and Head of the Section of Plant Pathology, Division of Pathology and Botany, University of Minnesota, University Farm, St. Paul, Minn.; Agent, Division of Plant Disease Control, Bureau of Entomology and Quarantine, U. S. Department of Agriculture, Washington, D. C.

William H. Taliaferro, Ph.D.
Eliakim H. Moore Distinguished Service Professor of Parasitology, Chairman of the Department of Bacteriology and Parasitology, and Dean of the Division of Biological Sciences, The University of Chicago, Chicago, Ill.

E. A. Watson, V.S.
Chief, Division of Animal Pathology, Science Service, Department of Agriculture, Ottawa, Ont., Canada

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THE NEED FOR RESEARCH ON THE GENETICS OF PATHOGENIC ORGANISMS

By E. C. STAKMAN

DIVISION OF PLANT PATHOLOGY AND BOTANY, UNIVERSITY OF MINNESOTA, ST. PAUL, MINN.; AND DIVISION OF PLANT DISEASE CONTROL, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, U. S. DEPARTMENT OF AGRICULTURE

THERE is urgent scientific and practical need for extensive investigation of the genetics of micro-organisms that cause diseases of man, domestic animals, and economic plants, for knowledge of the genetics of pathogens has lagged behind that of the organisms they attack.

Studies on the genetics of higher organisms have been motivated both by scientific curiosity and practical necessity. A vast amount of genetic research has been done to discover the laws and mechanisms of inheritance and to gain a better understanding of organic evolution. But much has been done also for the purpose of developing sound procedures in the improvement of crop plants and domestic animals. It is generally recognized that "improving the germ plasm of plants and animals" can be accomplished effectively and efficiently only by application of the principles of genetics. These principles are indispensable guides to plant and animal breeders, and they must be guides also to pathologists who are attempting to improve plants and animals through the control of certain kinds of diseases.

How much is known about the genetics of pathogenic micro-organisms? Far less than about the laws of inheritance in higher organisms. This may be due partly to the relative minuteness of most pathogens, with consequent difficulty in experimental manipulation and interpretation of results. But the blame can not be placed entirely on the micro-organisms; some of it attaches to microbiologists. However, this is not the place to indict ourselves but to take stock of what we know, what we need to know, and why we need to know it.

The problem of the genetics of pathogenic organisms is essentially the problem of their variation. That they do vary and that the variation sometimes is extremely important is common knowledge. But how, how much, and why do they vary? What are the limits of variation, both with respect to the kind and magnitude of the modified characters and with respect to their duration? Is the variation temporary or permanent; is it due to the effect of environmental factors or to genic changes either induced by the environment or independent of it? Can pathogenic micro-organisms adapt themselves to new environmental conditions merely by being subjected to them? Can pathogens change in virulence as a result of host influences? Can they increase in virulence as a result of successive passages through a given host? Is the change, if it occurs, quite temporary; is it in the nature of a *Dauermodifikation;* or is it permanent and heritable? Or are the apparent changes due merely to natural or conscious selection of strains from a mixed population; and do new strains arise as a result of mutation and hybridization, as in the higher plants?

Conclusions regarding the nature of variation in micro-organisms are justified only if the initial degree of genetic purity of the cultures is known. For this purpose neither species nor the pure culture, as the terms ordinarily are used, has any real meaning. It seems highly probable that erroneous conclusions often have been drawn because of lack of recognition of this fact.

We are just beginning really to appreciate what a species is. What a confederation of lines, strains, and varieties many species are! The cultivated Irish potato is mostly *Solanum tuberosum* L. But what an array of varieties within the species: white-flesh varieties and yellow-flesh varieties; red-skin and white-skin varieties; early varieties and late varieties; several hundred varieties differing in morphological or physiological characters or both. There are dozens or hundreds of varieties and strains of most crop plants. And this is not only because man has attempted to perpetuate varieties or lines within cultivated species. The more thoroughly species of wild plants are studied, the more evident it becomes that they also comprise many distinct varieties or strains. This is becoming very apparent in the work now being done to improve native grasses. Perhaps a good illustration is creeping bent grass, *Agrostis stolonifera* Hort., so extensively used for golf greens. A large number of strains were tested before relatively few were finally selected as being most suitable.

Two of the most beautiful demonstrations of strain differences within species of higher plants ever seen by the writer were with orchard grass, *Dactylis glomerata* L., and Scotch pine, *Pinus sylvestris* L. At the plant breeding station at Aberystwyth, Wales, investigators a number of years ago had selected from the vicinity a considerable number of plants of orchard grass and propagated them vegetatively. The remarkable differences between the resulting clonal lines and the relative uniformity of the plants within each line made an indelible impression regarding the composition of a species. Not only did the lines differ distinctly in morphologic characters, but some were so susceptible to stripe rust, *Puccinia glumarum* Erikss., that they were literally yellow with rust, while others were virtually immune. And in Germany the writer saw comparable plantings of Scotch pine, from seed of different sources, that were so distinct from each other that a zealous taxonomist could have described several species. One of the most striking differences was in reaction to the leaf-cast disease: Some lines were highly resistant and others completely susceptible. They differed also in other physiologic characters, including rate of growth, and in some morphologic characters; yet they had in common certain morphologic characters that distinguished them from all other species of pines.

It is, of course, banal to call attention to the number of races or breeds of domestic animals within a species: cattle, horses, sheep, swine, dogs. The breeds differ greatly in morphologic and physiologic characters. And yet, everyone can recognize a horse as a horse and a cow as a cow, although he may not know to what breed it belongs. But, if he wanted to start a dairy, he would be wise not to stock his farm with Herefords.

What has all this to do with pathogenic micro-organisms? It is a platitude that many species of pathogenic micro-organisms comprise numerous strains that may differ in cultural characters on artificial media, in morphology, in general physiologic characters, or in pathogenicity. This is illustrated well by many species of fungi pathogenic to plants, two of which may be selected as examples.

Puccinia graminis Pers. causes stem rust of wheat, oats, barley, rye, and more than a hundred cultivated and wild grasses. As early as 1894 Eriksson showed that, although the rusts from different plants were essentially alike morphologically, the rust from one kind of host might not necessarily infect another kind. Eventually, six "varieties" of *P. graminis* were recognized in North America. One of these, *P. graminis tritici* Erikss. and Henn., attacks wheat, barley, and certain wild grasses but not oats, rye, and certain other wild grasses. The *tritici* variety, therefore, can be distinguished easily from the *avenae* Erikss. and Henn. variety, which attacks oats and certain wild grasses but not wheat, barley, or rye. But each of these varieties in turn comprises physiologic races that can be distinguished by their parasitic effects on selected varieties of wheat and oats, respectively. The *tritici* variety comprises at least 180 distinct races that can be recognized by the degree of infection they produce on 12 varieties of *Triticum* spp., that is, wheat, einkorn, and emmer. Some of these races have been found in North America, Europe, and Asia; others in North America, Australia, and New Zealand. Regardless of their source, their parasitic effects are alike and distinctly different from those of other races. The species *Puccinia graminis* comprises six varieties, and some of these varieties in turn comprise many physiologic races. For taxonomic purposes, then, *P. graminis* means something: it means a pleomorphic rust fungus that has a certain life history and certain morphologic characters that distinguish it from other species of rust fungi. But for pathologic purposes, *P. graminis* is entirely insufficient: far greater refinement of analysis is essential. If one wants to know what effect the rust will produce on a given host, a variety of wheat, for example, he must deal with *P. graminis tritici* physiologic race 21, or one of the other 180 physiologic races so far identified. Race 21 attacks Marquis wheat and the durums but not Kanred. Race 56 attacks Marquis and Kanred but not the most commonly grown varieties of durum. The writer and some of his associates once isolated nine different races from a single collection of rusted wheat obtained in Egypt, and recently they have isolated more than 30 races from two barberry bushes (the alternate host of *P. graminis*). What spectacular and conclusive evidence could have been obtained for changes in virulence had a series of varieties of wheat been inoculated without knowing the technics for recognizing the different races of rust! And considerable evidence of the same nature could have been obtained had the host material not been relatively pure.

Ustilago zeae (Beckm.) Ung., the fungus that causes corn smut, is another good example of a species that embraces a large number of strains. It is almost literally true that nearly every monosporidial line that is isolated from nature differs somewhat from every other one. Several years ago the writer and several associates attempted to determine the number of strains within the species. Detailed evidence need not be given here, as many of the results have been published.

However, at least a dozen people have investigated certain phases of the problem at different times and all agree that the number of strains within the species is nothing short of astounding and would be almost unbelievable unless a person had had direct experience with them. Furthermore, it has been proved by means of breeding tests that these lines are neither temporary variants nor ecads, but are genotypically different from one another (Stakman *et al.* 1933). The only conclusion that can be drawn regarding so ordinary a plant pathogenic fungus as *Ustilago zeae* is that the species comprises an indefinite number of biotypes that differ from one another in many characters, including cultural characters, relation to temperature, biochemical effects, sex, and pathogenicity. And yet all the biotypes have certain characters that justify including them within the species. If one examined merely the chlamydospores and based his conclusions on their size, shape, and wall characters, he would conclude that *U. zeae* was a relatively uniform and simple species. But he would be very much in error.

What, then, is a species? In the writer's experience, it is a group of biotypes that can be distinguished with reasonable certainty from other groups of biotypes. Theoretically, a species might comprise a single biotype, but the writer has not had experience with such a species among plant pathogenic fungi. Literally, there are almost innumerable biotypes in some of the species. This fact is interesting, and it is also extremely important. Certainly in practical work with plant diseases it is necessary to know the number and the capabilities of biotypes within a species; and gross errors would be made in attempting to interpret many important pathological phenomena without such information.

''Biotype'' may be more or less of a philosophical abstraction, as it connotes a group of individuals with exactly the same genetic constitution, and the difficulties of proving identity of genetic constitution are obvious.

But yet, certain biotypes, physiologic races of *Puccinia graminis* for example, have been maintained for more than ten years and their behavior throughout that period has been remarkably consistent. If there are many biotypes within a species, differing principally in physiologic characters, including pathogenicity, what constitutes a pure culture and how can it be obtained?

The implication of the term "pure culture" is that it comprises a homogeneous group of individuals, somewhat like a pure line. However, this definitely and distinctly is not true of many so-called pure cultures. For genetic purposes, and for many practical purposes, a pure culture may be highly impure. Ordinary dilution cultures can have but relatively little standing in genetic investigations. Even a monosporous culture may contain several biotypes. This is well exemplified by many of the smut fungi. A smut culture derived from a single chlamydospore is likely to contain at least four biotypes. This was not known before the sexual nature of the chlamydospore was discovered. Chlamydospores are diploid, resulting from the fusion of nuclei of opposite sex, which often differ also in factors other than those for sex. When the chlamydospores of such smut fungi as *Ustilago zeae* germinate, therefore, they produce a promycelium on which four haploid sporidia are normally formed. These sporidia may be of four kinds on the basis of all characters which can be studied in monosporidial cultures obtained from them as a result of vegetative budding. What conclusions could, therefore, be based on studies on monosporous cultures of the smut fungi? Such cultures almost surely would contain several haploid biotypes, sexual fusions between different kinds of nuclei precedes the formation of chlamydospores in inoculated host plants, recombination and segregation could occur; and the lines obtained from the chlamydospores produced on the inoculated plants might be entirely different from those that were inoculated into the plants.

The conidia of many of the Fungi Imperfecti are multinucleate and in many cases the origin of the nuclei is unknown. It is quite possible that if the nuclei contain different genetic factors there may be chance assortment in cultures derived from single spores; thus, starting with a single spore, a person may wind up with a group of biotypes when he expected to have only one. If conclusions regarding variation are to be drawn from the performance of a culture derived even from a single cell, the nuclear condition and the potentialities of the cell must be known. Otherwise, there is no justification for concluding that there has been heritable change, because the change may be due to the selective effect of the environment on the biotypes in a mixed population.

Plant pathogenic fungi have been accused of doing some queer things. But why should they not do queer things when they often are a queer mixture of a number of queer biotypes?

It is not always easy to determine the nuclear condition of even such relatively large objects as many fungus spores. It is true that the nuclei usually can be seen and their number determined, but it often is virtually impossible to determine the number of chromosomes, and consequently the haploidy or diploidy of the nucleus, because of their relatively small size. The cytological events preceding the formation of a particular kind of spore must be known and understood and the behavior of the particular kind of spore must be determined before one can know what to expect of cultures derived from it.

How much can be said for pure cultures of bacteria? Most studies on bacteria causing diseases of plants, human beings, and other animals have been made with cultures obtained by the dilution method, often from many individuals. In some recent investigations single individuals have been isolated, but, even so, what is the genetic constitution of a single individual? Is the life history of bacteria as simple as it seems? Or are the bacteria pleomorphic and do some of them have relatively complex life cycles? That many species of bacteria comprise strains or races just as do the fungi has been demonstrated repeatedly. Where did these strains come from? How many of them were present in a so-called pure culture? And unless there is assurance that several of them were not present in a "pure culture," what justification is there for assuming that a given organism changes decidedly in response to its environment? Suppose that inoculations were made with a culture containing a number of biotypes or strains or races, if those terms are preferred to the term biotype. How much change is there likely to be in the mixed population as a result of exposing it to different environmental conditions? How are attenuation, increase of virulence, or the development of resistance to chemicals to be interpreted? There is a vast difference, both scientifically and practically, between changing the potentialities of a given biotype and in so manipulating a culture as to favor or inhibit the development of certain component biotypes.

That apparent increase in virulence as a result of host influence sometimes is due merely to perpetuation of certain biotypes and the elimination of others is shown conclusively by experience with certain plant pathogenic fungi. This can be illustrated with many of the rust fungi and smut fungi. If the differential hosts used for determining physiologic races of *Puccinia graminis tritici* are inoculated with a collection of rust, the following results may appear on the varieties of wheat listed below:

Little Club	Heavy, abundant infection
Marquis	Heavy, abundant infection
Kanred	A few pustules only
Arnautka	Virtually immune

If the rust is then transferred again from Marquis, the same result would be obtained. If, however, it is transferred from the few pustules on Kanred, there may be the following results:

Little Club	Heavy, abundant infection
Marquis	Resistant
Kanred	Heavy, abundant infection
Arnautka	Heavy, abundant infection

It easily could be concluded that the rust had changed its virulence on Kanred. But this is not what has happened. In the original inoculum there was a small amount of race 38 and a very large amount of race 49; race 49 does not attack Kanred, but race 38 does. Race 49 does not attack the durums normally, and race 38 does. As Marquis is moderately to highly resistant to race 38, the few pustules of this race could be obscured by the heavy development of race 49. The rust on Kanred, however, would be pure 38, and when transfers are made this race would not have to compete with the others. While it would appear, therefore, that the rust has lost virulence for Marquis and has gained it for Kanred, as a result of its sojourn on Kanred, the fact is that Kanred has merely eliminated one biotype from the mixed population and has permitted the development of the other. It would be rash to assert that all experiences of this type with all pathogenic micro-organisms are due to causes similar to those just given. On the other hand, this is exactly the type of thing that happens so commonly that it is essential to eliminate the possibility before being too sure that the pathogen actually has changed.

The cardinal fact that cannot be emphasized too strongly is that one must know what is in his cultures, even though he calls them pure cultures, before assigning the causes for certain peculiar behaviors. This is not merely theoretical: it would be possible to adduce an overwhelming mass of evidence to show that many misinterpretations have resulted from just such situations as have been described above. It may be impossible in many cases to know the cytological condition of single cells from which cultures are obtained, as is the case with bacteria, for example. But if the cytological conditions cannot be determined, it would be wise not to draw sweeping conclusions regarding the nature of what has happened. The facts may be clear, but the interpretation of those facts easily can be quite erroneous.

It must be clear from the above that many apparent changes in the capabilities of micro-organisms probably are not changes in the sense imputed by such terms as attenuation, increase in virulence, and adaptation. They may result merely from the selection or reassortment of biotypes in a mixed population. And many of these biotypes differ appreciably in physiological characters only, not in morphological characters. Consequently, the demonstration of their existence is difficult unless the proper differential media, differential hosts, or other differential agents are discovered.

But if cultures comprising single biotypes can be isolated and propagated, is there still possibility of change? Single biotypes will, of course, vary under different environmental conditions. This phenotypic variability, however, probably is so clearly understood that it would be superfluous to discuss it at length. Nevertheless, it may be well to remember that pathogenic micro-organisms often are grown under very unnatural conditions, and the variability may, therefore, be very great. Alpine forms of the ordinary trembling aspen are likely to be quite different in size and general habit from plants of the same species grown in rich soil at lower elevations. Such variability is quite obvious to the eye. The variability of micro-organisms can be equally great, but the difficulty of distinguishing between phenotypic variability and genetic variation also is sometimes very great. Consequently, it is important to determine the limits of phenotypic variability. There is a more important question, however. Do the results of this variability ever become fixed more or less permanently in the individuals of a biotype? Is the cytoplasm or the germ plasm of the organism changed sufficiently by the environment to be more or less permanent? Do *Dauermodifikationen* occur?

Whether micro-organisms acquire new and semi-permanent or permanent potentialities as a result of environmental conditions, including host influences, has long been an important question. A number of years ago it seemed to have been settled for certain plant pathogens, but it has again become decidedly unsettled. There was a time when the researches of such eminent mycological investigators as Marshall Ward (1903), Salmon (1904), Freeman and Johnson (1911), and others seemed to have proved that certain plant pathogenic fungi could adapt themselves to resistant plants merely as a result of successive transfers to those plants or to "bridging hosts." According to these investigators, if plants A and B were inoculated with a given rust, with A susceptible and B highly resistant, it was possible to "educate" the rust to infect B normally, merely by making successive transfers. In many of the experiments the time required was short, a few transfers sufficing to enable the rust to adapt itself to B. There seemed to be conclusive evidence also that if species or varieties A, B, and C were inoculated with the rust, with A and B susceptible but C resistant, the rust might acquire the ability on B to attack C. Host B would, therefore, be a bridging host, the assumption being that it was intermediate taxonomically between A and C. A theoretical explanation might be that the rust accumulated certain growth-promoting substances on B which were absent in C and which, therefore, might be stored in sufficient quantity in the pathogen to enable it to grow on C. The difficulty with even this theoretical explanation is that growth-promoting substances should have been present in A as well as in B if the rust grew normally on both of them. Furthermore, since the newly acquired ability to grow on C was supposed to be relatively permanent, either C or the pathogen would have to produce the necessary substances after the supply obtained from B had been exhausted.

However, theoretical refutation of the theory of bridging and adaptation is not necessary, because abundant experimental evidence has been obtained that rusts do not behave in the manner indicated, provided a single biotype is used in the experiments. The writer and others made very extensive experiments with *Puccinia graminis* and never obtained the slightest evidence of adaptation or bridging. They found, however, that there were many different parasitic races of some of the rusts and that "adaptation" and "bridging" were easily explicable on this basis.

Recently, certain experiments by Reddick and Mills (1938) seem to indicate that the late-blight fungus, *Phytophthora infestans* (Mont.) DeB., may "adapt" itself to resistant varieties of potatoes. However, Müller (1935), in investigating the same problem, could obtain no evidence of this type of effect. A dogmatic statement would be unsafe and unscientific, but there is a mass of evidence that adaptation and bridging do not occur to any considerable extent, at least in some plant pathogens.

If micro-organisms can adapt themselves to new environments, it ought to be possible for them to develop tolerance for germicides and other chemicals. There is some evidence that they actually do. The work of Jollos (1921) on the production of *Dauermodifikationen* in certain protozoa furnishes evidence that paramecia acquire the ability to tolerate increasing concentrations of arsenic if grown on media in which the amount of arsenic is gradually increased. This development of a certain ability in a certain direction, merely by creating the environment to which one wishes the organism to adapt itself, is highly significant if true. Many attempts to control diseases might then defeat their own purpose. Disease-resistant varieties of plants would not remain resistant very long, because the pathogen would adapt itself to them. In human and animal pathology, chemotherapy might in the long run defeat its own purpose, because it would merely result in the acquisition by the pathogen of resistance to the chemicals used. Is it possible that the explanation for observed facts is different from that often given? Critical researches on the subject are urgently needed. Again, the writer would like to emphasize the fact that little reliance can be placed on the interpretation of results on variation unless the genetic purity of the organism involved is assured. But can it ever be assured? Even if experiments are started with single biotypes, that is, pure lines of the micro-organism, is mutation likely to occur?

Mutation is almost unbelievably common in certain fungi. Probably the surest method of getting a single biotype of a plant pathogenic fungus would be to isolate a single sporidium of one of the smut fungi. It consists of a single cell, is usually haploid, and propagates vegetatively by budding in a yeast-like manner. And yet one can have assurance that a culture resulting from a monosporidial isolation is a single biotype only if there is no mutation. There is an overwhelming mass of evidence that hundreds of mutants can be isolated within a relatively short time from cultures of what originally comprised a single biotype. While the mutations usually result in the loss of one or more characters, there sometimes appears to be a gain, either direct or indirect. If the mutation is due to chromosomal aberration, it is quite possible for the mutant to acquire additional ability as a result of gaining part of a chromosome or as a result of chromosomal interchange, with consequent changes in the relative positions of genes, or through the loss of an inhibitor. However, theoretical speculations are not so important as facts for present purposes. The really important fact is that mutation has been demonstrated to occur with such frequency in many fungi that caution is advisable in drawing conclusions regarding adaptive changes. It is true that certain environmental factors may stimulate mutation, but the mutants are not necessarily better adapted to the environment which stimulated their creation. Obviously, if there are several mutants in a new environment, only those fit to survive in that environment will survive, and the others will be eliminated. It may appear, therefore, that the environment has exerted a directive influence; and in one sense it has, but certainly not in the sense ordinarily imputed by such words as education and adaptation.

One of the papers in this symposium deals specifically with the subject of mutation; therefore, further details will not be given here. The writer does not wish to be understood as even intimating that mutation accounts for all of the observed facts in connection with changes in cultures of pathogenic micro-organisms. He does wish to be understood, however, not only as saying but as insisting that the degree of mutability of an organism must be known before it is safe to conclude that it has been changed by the environment in such a way as to enable it to live more successfully in that environment. There is a wide difference between believing that an organism can be bent to the will of man by subjecting it to certain environments and believing that certain environments may induce genetic changes the products of which may or may not be better suited to the new environment.

Loss in virulence of certain organisms on culture media and the regaining of virulence on appropriate hosts often have been recorded. This could conceivably be due to the presence of growth-promoting substances in the host plant but not in the culture medium. Since these substances may be effective in very minute quantities, it is quite possible that the pathogenic phase of the organism might store up enough to enable it to grow saprophytically until the supply of the substance in question had been exhausted. It would then be necessary to grow the organism in the host long enough to enable it to replenish its supply. This is a theoretical possibility and may be fact. However, the writer knows of abundant evidence to indicate that apparent loss in virulence sometimes has been due to the production of fast-growing relatively nonpathogenic mutants. The fast-growing mutant may suppress the original virulent biotype to such an extent that very little of the original is left in the culture. One or more transfers to the appropriate host would obviously tend to eliminate the less virulent mutant and permit the more virulent type to increase. On isolation, therefore, the original virulent biotype would be recovered and there would be strong likelihood that the less virulent one might be lost completely.

Obviously, one of the very important causes of genetic variation is hybridization. Unfortunately, little or nothing is known about sexual stages in some pathogenic micro-organisms; possibly sexual reproduction does not occur in some of them. On the other hand, it definitely is of the greatest importance in some cases. Some of the fungi that cause diseases of plants are pathogenic only in the dikaryophase, that is, after plasmogamy and before karyogamy. In general, the smut fungi, for example, cause infection only after lines of opposite sex have united. This is the subject of a special paper in this series, and it is unnecessary, therefore, to give details. However, as evidence of the number of biotypes that can result from fusions between haploid monosporidial lines, the writer and his associates once crossed two such lines of *Ustilago zeae* and obtained about 40 segregates from the resulting hybrid, no two of which were alike, not even the parental types being recovered.

It seems scarcely necessary to emphasize the practical importance of more extensive and precise knowledge regarding the genetics of pathogenic micro-organisms. Such knowledge is essential, in many cases, in understanding the course of development of epidemics. It is important in vaccine therapy, probably often in sero-therapy, in chemo-therapy, and, in the case of plant diseases, it is of the greatest importance in the breeding of resistant varieties, and probably is important also in some cases in control by the use of fungicides. The whole subject is fascinating scientifically and is so important practically that it should be investigated basically and thoroughly. In attempts to control those diseases caused by micro-organisms, the aim is to eliminate the pathogen or to prevent its destructive development. Surely this can be done best if the limits of variability and variation of the pathogen are known.

References Cited

Ark, P. A. 1937. Variability in the Fire-blight Organism, Erwinia amylovora. *Phytopath.*, 27: 1.

Christensen, J. J. 1929. Hybridization. Pt. II, *Minn. Agr. Exp. Sta. Techn. Bull.*, 65: 89.

Eriksson, J. 1894. Ueber die Specialiesirung des Parasitismus bei den Getreiderostpilzen. *Ber. Deut. Bot. Gesell.*, 12: 292.

Freeman, E. M. and Johnson, E. C. 1911. The Rusts of Grains in the United States. *U. S. Dept. Agr. Bur. Plant Ind. Bull.*, 216.

IVANOFF, S. S., RIKER, A. J. and DETTWILER, H. A. 1938. Studies on Cultural Characteristics, Physiology and Pathogenicity of Strain Types of Phytomonas stewarti. *J. Bact.*, 35: 235.

JOLLOS, V. 1921. Experimentelle Vererbungsstudien an Infusorien. *Zeitschr. f. Indukt. Abstammungs- u. Vererbungslehre*, 24: 77.

KNIEP, H. 1929. Vererbungserscheinungen bei Pilzen. *Bibliogr. Genetica*, 5: 371.

McNEW, G. L. 1937. Isolation of Variants from Cultures of Phytomonas stewarti. *Phytopath.*, 27: 1161.

MÜLLER, K. O. 1935. Über den augenblicklichen Stand unserer Kenntnisse zur biologischen Spezialisierung des Krautfäuleerregers der Kartoffel. *Züchter*, 7: 1.

PATEL, M. K. 1928. A Study of Pathogenic and Non-pathogenic Strains of Pseudomonas tumefaciens Sm. & Town. *Phytopath.*, 18: 331.

REDDICK, D. and MILLS, W. 1938. Building up Virulence in Phytophthora infestans. *Amer. Potato J.*, 15: 29.

REED, G. M. 1935. Physiologic Specialization of the Parasitic Fungi. *Bot. Rev.*, 1: 119.

SALMON, E. S. 1904. On Erysiphe graminis DC. and Its Adaptive Parasitism within the Genus Bromus. *Ann. Myc.*, 2: 255.

STAKMAN, E. C., PIEMEISEL, F. J. and LEVINE, M. N. 1918. Plasticity of Biologic Forms of Puccinia graminis. *J. Agr. Res.*, 15: 221.

STAKMAN, E. C. 1929. Physiologic Specialization in Pathogenic Fungi. *Proc. Intern. Congr. Plant Sci., Ithaca*, 2: 1312.

————. 1936. The Problem of Specialization and Variation in Phytopathogenic fungi. *Genetica*, 18: 372.

————. 1938a. Plant Disease Fungi Constantly Evolving New Types. *Science*, 88: 438.

STAKMAN, E. C., CHRISTENSEN, J. J., EIDE, C. J. and PETURSON, B. 1929a. Mutation and Hybridization in Ustilago zeae. Pt. I, *Minn. Agr. Exp. Sta. Techn. Bull.*, 65: 3.

STAKMAN, E. C., TYLER, L. J. and HAFSTAD, G. E. 1933. The Constancy of Cultural Characters and Pathogenicity in Variant Lines of Ustilago zeae. *Bull. Torrey Bot. Club*, 60: 565.

STAKMAN, E. C., LEVINE, M. N., CHRISTENSEN, J. J. and ISENBECK, K. 1935. Die Bestimmung physiologischer Rassen pflanzenpathogener Pilze. *Nova Acta Leopold.*, 3: 281.

STAKMAN, E. C., CHRISTENSEN, J. J. and BECKER, HANNA. 1938. Pathologische Probleme bei der Züchtung krankheitswiderstandsfähiger Weizen- und Gerstensorten im Sommerweizengebiet der Vereinigten Staaten von Amerika. *Züchter*, 10: 57.

WARD, H. M. 1903. Further Observations on the Brown Rust of the Bromes, Puccinia dispersa (Erikss.), and Its Adaptive Parasitism. *Ann. Myc.*, 1: 132.

GENETICS OF VIRUSES PATHOGENIC TO ANIMALS

By E. A. WATSON

SCIENCE SERVICE, DEPARTMENT OF AGRICULTURE, OTTAWA, ONTARIO, CANADA

THE question: What is a virus?, is a familiar one, frequently put and still awaiting answer. Are viruses living or dead, animate, inanimate, particulate, biological or biochemical elements, agents, transmitters, enzymes, ferments, catalysts, or what? Without knowing what a virus truly is, considerable knowledge regarding the properties and characteristics of some of the viruses has been gained. It can be said that a virus is a filterable, infective agent, invisible under the microscope; hence the term ultramicroscopic, filterable viruses. "Filterable" can have only a relative sense and meaning, being dependent upon the nature of the filter and its density, the physical properties of the fluid material, electric charges, and other factors. Moreover, some micro-organisms have filterable phases or forms in their life cycles. The belief that viruses are living agents rests mainly on the fact that they are capable of multiplication and adaptation in the living body and, *in vitro,* in the presence of living cells. Some viruses are infective for susceptible animals in very high dilutions. They are titratable and the minimum infective dose can be determined by tests on suitable laboratory animals according to standardized technique. When an animal or a living chick embryo is infected with 1 to 10 minimal doses, with ensuing fatal results, it is found that the virus has reproduced itself in relatively enormous quantities, a small fragment of tissue yielding billions of infective doses. Perpetuation of the virus and its multiplication at each successive animal passage can be carried forward at will for unnumbered generations or passages. Animals which have recovered from a virus disease or which have survived experimental infection with a virus of modified or attenuated activity are more or less immune to subsequent infection with the same virus. In such cases the serum may contain anti-viral bodies capable of neutralizing the activity of the corresponding virus *in vivo* and *in vitro.* A striking feature of many of the viruses is their power of adaptation to foreign hosts and changed environment. Evidence, so briefly indicated in the foregoing, of reproduction, multiplication, adaptation and the development of anti-viral bodies and immuno-biological reactions—corresponding to similar evidence in respect to living bacteria—forms the basis of belief in the living nature and particulate state of viruses from animal sources. But no virus from such sources has yet been obtained in a pure state or dissociated from its coexistence with and dependency upon the complex proteins and molecules of living tissue cells. Until that has come about by cultivation in artificial media in the absence of living matter and more exact criteria are available for determining the size, structure and physical properties of viruses, their origin, nature and mode of development lie in obscurity and are open to doubt and question, and to new ideas and conceptions.

Certain virus diseases are associated with the formation of bodies generally referred to as cell inclusions, or virus bodies, occurring in the cell cytoplasm or in the cell nucleus or in both cytoplasm and nucleus. They are more or less pleomorphic, of various kinds and types, differently situated within the cell and peculiar to certain types of cell. Among the best known examples there may be mentioned (1) Negri bodies, occurring within the ganglion cells of the brain, particularly in Ammon's horn, of animals affected with rabies; (2) Guarnieri bodies, within the epidermal cells in lesions of vaccinia and sheep pox; and (3) Bollinger bodies, within the epidermal cells of lesions of fowl pox. The nature, significance and relationship of these and other like inclusion bodies to the specific virus with which they are associated is a much discussed question. Their presence or absence may be of much diagnostic importance. Some authorities are inclined to believe that the inclusion bodies and the virus are one and the same, or that the inclusions are bodies which produce the virus. Others view the inclusions as degeneration products of cells attacked by virus. They are not to be observed in all virus diseases but, in fact, only in the minority. They are found mostly in association with neurotropic and dermotropic viruses; and but rarely and doubtfully in typical septicaemic and viscerotropic virus diseases.

Selective affinity for one of the body tissues of its host is a striking characteristic of certain viruses as, for example, the neurotropic viruses of rabies, equine encephalomyelitis and louping-ill of sheep, and the dermotropic viruses of pox diseases. In these the viruses appear to multiply only in the tissues attacked: the brain and spinal cord, the skin and mucous membranes, according to the selective affinities of the respective viruses. On the other hand, in the virus diseases that occur as acute, generalized infections, such as hog-cholera, South African horse-sickness, infectious equine anaemia, distemper and influenza, the causative viruses multiply in the circulating blood and viscera and are there found in greatest concentration.

There do not appear to be any significant differences between virus and bacterial diseases in the manner of their occurrence and their wide range and variability in respect to contagiousness, infectivity, points of attack, clinical manifestations, selectivity and immunity. As regards allergy and hypersensitivity to virus disease, little, if anything, is known and the question does not appear to have been explored, owing, probably, to the difficulties in preparing suitable test reagents.

Ample evidence has been produced to show that a specific virus disease can be caused by strains or types of virus immunologically distinct. This is well recognized and exemplified in foot-and-mouth disease which is caused by at least three virus strains, in South African horse-sickness with its six or more virus strains, and in equine encephalomyelitis with its so-called Eastern and Western types or strains. Recovery from a specific virus infection leaves more or less immunity against reinfection with the same virus. But where the virus is represented by a plurality of strains, high protection or solid immunity prevails against only one strain. Protection will be slight or, at best, only partial against other strains of the same group. A solid immunity against the homologous strain may therefore prove ineffective against heterologous strains. The coming into existence and development of immunologically distinct strains of a virus may well be conceived as being brought about by changed environment, animal host species and mode of adaptation. Knowledge of the multiplicity of virus strains is growing and it is probable, as investigations continue, that a much greater number than at present recognized will be uncovered.

One of the greatest handicaps in the study of viruses has been the difficulty in transmitting them and propagating them in laboratory experimental animals. But this is being overcome with increasing knowledge of the susceptibility of the guinea pig, rabbit, white mouse and ferret to certain virus infections and of the behavior of such viruses in these animals and in the developing chick embryo. Even now there may be more or less difficulty in making the initial transfer from the natural to the experimental host and in obtaining the first "take," which may cause little health disturbance or escape observation. But after a few serial passages the virus adapts itself to the new host and environment with, as a rule, increasing pathogenicity. With some viruses and experimental animals only a few passages or generations are required, and with others many, for the attainment of fixed virulence and type. With certain viruses, such as those of South African horse-sickness, and yellow fever of man, the phenomenon of changing a typical viscerotropic into a fixed neurotropic virus occurs as a result of intercranial inoculations in serial passage, particularly in white mice. Moreover, it would appear that the type of virus which has been developed by adaptation and fixation in the experimental host is maintained and is not reversed when reintroduced to the original host. Further modification is indicated by the observation that a virus, which has attained its highest possible virulence in the passage animal and kills it in a given period of time, is generally less infective and pathogenic for its original host than before its change of type and environment.

In our own distemper studies, virus strains originating from the fox, mink and dog have been maintained for a number of years by continuous serial passage in ferrets. Some minor differences in their infectivity and pathogenicity were apparent at the time they were first obtained, but none could be observed after serial passage in ferrets. And, while the strains are evidently fixed at their highest virulence for ferrets, these animals invariably succumbing to inoculation between the 10th and the 14th day following, usually between the 11th and the 13th day, it is rarely possible to produce clinical distemper with them in either dogs or foxes which were raised under strictly controlled conditions and which, theoretically, should be uniformly and highly susceptible to the disease. Thus, viruses that change in type and reach highest virulence in the passage animals may, at the same time and in the same process, become greatly attenuated for the original host species. Much practical use is being made of this in the preparation of living, attenuated viruses for prophylactic and immunization purposes from the brain tissues of white mice and the embryonic tissues of the developing chick.

Experimenting with the virus of equine encephalomyelitis, we succeeded after several failures in establishing it in guinea pigs by intracranial inoculations of naturally infected horse brain. After a few passages it attained high virulence and appeared fixed as a neurotropic virus for guinea pigs. By similar procedure the guinea pig strain was established and fixed in white mice and, in turn, the mouse strain in the chick embryo. The comparative infectivity and virulence of the three strains so developed in the guinea pig, mouse and chick embryo, respectively, are of significance. Titrating the virus-containing tissues of these animals in dilutions carried to 1:100,000,000 using 0.1 cc of each dilution as the volumetric dose for inoculation, the minimal infective dose of the guinea pig virus is represented as 1:1000, the mouse virus as 1:100,000 and the chick virus as 1:100,000,000. That is to say: the guinea pig virus has 50–100 times greater concentration and infectivity than the horse virus, the mouse virus 100 times that of the guinea pig virus, and the chick virus 1000 times that of the mouse virus. However, such striking differences are probably due to qualitative as well as quantitative factors; and the former, more difficult to put in evidence and to recognize, may be of no less importance in virus investigations, especially in their relationship to antibody production, serum-virus neutralization and immunity reactions, than the latter. It has already been demonstrated that the chick embryo strain of encephalomyelitis virus can be utilized for immunization purposes far more effectively than the other strains. But whether this is due alone to the great concentration of the virus in the chick embryo or to the concentration together with a change in the character of the virus itself, acquired by sojourn in the chick embryo, is at present an open question.

One more virus disease may be mentioned in concluding this short discussion, namely, infectious equine anaemia, which has points of special interest. It is a highly infective but non-contagious disease, septicaemia-like in character, appearing in either an acute or chronic form and marked by recurring febrile periods which, in the early stages, take place at remarkably regular intervals of approximately 21 days and are suggestive of a cycle not unlike that of certain protozoan infections. The virus is present and apparently multiplies in the circulating blood and has no known special affinities for cellular tissues. The minutest quantity of blood will transmit infection and disease to a healthy horse, and this, with other observations, has led to the strong suspicion that an insect host or vector is responsible for the natural transmission of the disease.

Horses which recover from the disease, and they are many, are apparently immune from further attack but may carry the virus in their blood for the rest of their natural lives and thus become virus reservoirs. As an instance, a horse which had recovered from the naturally contracted disease when about five years of age, lived the remainder of its life at one of our research stations, passing 20 years of age. At intervals during that whole period its blood proved highly infective for horses and reproduced the typical disease. The fact that apparently normal healthy horses may be and frequently are carriers of infectious-anaemia virus is not to be overlooked when horses are used for the production of antisera intended for either human or animal use.

Inability to infect any species of laboratory animal or to propagate the virus of infectious equine anaemia under laboratory conditions has hindered the investigations we have carried on for 25 years. But with improved methods of study recently introduced and experience gained with regard to susceptibility of mouse, ferret and chick embryo to virus infections, there are increasing possibilities of propagating this virus in the laboratory and of studying it to better effect.

Many other diseases in which the etiological agent is filterable and ultramicroscopic occur in North America. Many more, unknown on the North American Continent, are frequent in either Europe, Asia or Africa. These virus diseases are just as important, economically, as the diseases of known bacterial origin, with which they have much in common. The view that viruses are animate, particulate elements and agencies appears to be well taken and doubtless will be held until satisfactory evidence to the contrary has been produced. Their orderly classification, powers of multiplication and adaptation, as well as other inherent properties and affinities will for a considerable time to come hold the attention of virologists and, perhaps, the interest and scrutiny of geneticists.

GENETICS OF VIRUSES PATHOGENIC TO PLANTS

By L. O. KUNKEL

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY, THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, N. J.

RESEARCHES on virus diseases of plants have usually included studies on the behavior of the viruses as disease-producing agents. Except for statements that they may be passed in series, little mention has been made of either the progenitors or the progenies of plant viruses. The possibility of investigating the genetics of these agents was not seriously considered ten years ago. Although the existence of variant strains of different virus diseases has been recognized for some time (Bennett 1930; Carsner 1925; Carsner and Lackey 1928; Johnson 1926; Kerling 1933; Kunkel 1932; Lackey 1929, 1929a; Storey and McClean 1930; Storey 1931, and Thung 1932), not much had been learned until recently regarding the origin and nature of the strains. The work on mutations in tobacco-mosaic virus (Jensen 1933; McKinney 1926, 1929), cucumber-mosaic virus (Hoggan 1935; Price 1934), potato-X virus (Böhme 1933; Salaman 1933), and a few other viruses has served to focus attention on the frequency with which variants arise. It has also served to show the nature of variant strains and the limits within which they seem to be confined. I shall attempt to summarize briefly what is known regarding the origin and nature of strains of tobacco-mosaic virus and to report the results of studies on the frequency with which certain kinds of strains appear in the progenies of this and closely related viruses.

As is well known, tobacco-mosaic virus multiplies at a rapid rate in susceptible cells. Large numbers of particles, which we assume to be exactly like those that were present in the original inoculum, because they are capable of causing typical tobacco mosaic, are produced. When serial transfers are made to tobacco plants, the disease obtained remains unchanged regardless of the length of the series, provided a fairly large dose of virus is used in making each transfer. If, however, a minimal infective dose is used at each transfer, the series can not be carried indefinitely without change in the type of disease produced. Sooner or later tobacco mosaic will be lost and a new type of disease will appear. The new disease will be caused by a virus which can be shown by serological and immunological tests, as well as by pathological studies, to be closely related to the virus of tobacco mosaic.

Experiments have demonstrated that, when virus in juice from a diseased tobacco plant that has been inoculated with tobacco-mosaic virus only and has shown symptoms for about one month is, after proper dilution, plated out on leaves of *Nicotiana glutinosa* L. and subinoculations are subsequently made to healthy tobacco plants from the individual lesions that develop, an average of about one plant in each 200 inoculated will come down with a disease that is distinct from tobacco mosaic. The other plants will come down with typical tobacco mosaic. If the juice from which platings are made is from a plant that has been diseased for only a short period of time, the subinoculations may give less than ½ per cent of variants. If it is from a plant that has been diseased for several months, they may give 1 or 2 per cent of variants. The variants obtained by this minimal dose method of inoculation are of many different kinds. Some are diseases that are much milder than tobacco mosaic, others diseases that are more severe; some are green mosaics, others yellow mosaics; some spread rapidly through the plant tissues, while others spread very slowly.

The results are interpreted as evidence that a small percentage of the virus particles produced in sick plants are unlike those used to inoculate the plants. It seems evident that the tobacco-mosaic virus particle gives rise to two types of progenies, a large progeny that is apparently exactly like itself and a smaller progeny that is distinctly different. As virus is passed from plant to plant by ordinary methods of inoculation, the unlike progeny is overgrown by the like progeny and the disease type remains constant indefinitely. This results apparently from the constancy of the selective environment rather than from lack of variability in the progeny of tobacco-mosaic virus.

In order to survive and produce disease, the variants must be isolated from the virus of tobacco mosaic. It should be clearly understood that the method used to isolate variants has nothing whatever to do with their production. They arise repeatedly as the tobacco-mosaic virus multiplies in sick tissues. We know of no means of either hindering or facilitating their production. They have not yet been shown to arise *in vitro*.

Several methods have been used in the isolation of variant strains of tobacco-mosaic virus. McKinney (1926) was the first to show that yellow variants may be obtained from bright yellow spots of irregular sizes and shapes that occur repeatedly in mottled tobacco leaves. By needle-puncture transfers, Jensen (1933) isolated a large number of yellow variants from such spots. Holmes (1934) isolated mild strains of tobacco mosaic by holding tomato tissues in which ordinary tobacco-mosaic virus was multiplying at a temperature of about 35° C. The high temperature apparently favored the multiplication of certain variants that caused mild diseases. The fact that such variants were not invariably obtained suggests that they did not result from attenuation of tobacco-mosaic virus. Carsner and Stahl (1924) showed that the virus of a mild strain of curly top of beets could be obtained by passing the ordinary strain through the weed *Chenopodium murale* L. Insofar as I am aware, no passage strain has been obtained from tobacco-mosaic virus directly, but many have appeared in tobacco plants infected with slow-moving strains of this virus.

Experiments have shown that tobacco-mosaic virus mutants may be passed in series, but that most of them can not be passed indefinitely without change, even when large doses of virus are used at each transfer. Sooner or later they are replaced by diseases similar to or identical with tobacco mosaic. In this respect they differ sharply from the latter. A few of the new viruses have, however, remained unchanged after a long series of transfers. Further work will show whether or not they are as stable as tobacco-mosaic virus.

Little is known regarding the influence of host plants on the production of virus mutants. Bright yellow spots from which yellow mutants may be isolated occur about as frequently in any unit area in the leaves of tomato and petunia plants affected by tobacco mosaic as they do in leaves of tobacco plants similarly affected. The yellow mutants obtained from these plants are similar to those from tobacco. We do not have much information on this point, but the little we do have suggests that the host cell is not a determining factor in the production of variants.

There are many things we wish to know regarding the unlike progeny from tobacco-mosaic virus. We would like to know in what respects it differs from the parent strain and of how many distinct types of virus it is composed. Do the same mutants arise again and again, or does each one differ from all the others? Do the mutants give rise to submutants and, if so, do they all give rise to the same kinds? Are any of the mutants so different from tobacco-mosaic virus that a relationship with the latter is questionable? Can the new diseases maintain themselves in nature? Will any of them affect plants that are immune from tobacco mosaic? Can they be transmitted by insects?

Too little is known at the present time to give satisfactory answers to most of these questions. We have isolated 130 variant strains of tobacco-mosaic virus and have passed them in series in Turkish tobacco. Many of the viruses in this group are similar and some appear to be identical. Nevertheless, further work may show that each one is different from all of the others. No two that have been studied carefully have proved to be exactly alike. The mutants differ from tobacco-mosaic virus in many ways. They differ in the severity of the diseases produced (Jensen 1933), in the extent to which they approach the tips of plants (Holmes 1936), in the rate at which they invade tobacco tissue, in whether or not they are capable of causing generalized infection, in the character of the primary and secondary lesions produced, in whether or not they mottle stem tissues, in infectivity, in stability, and in immunological and serological characteristics.

Many of the mutants have given rise to submutants and these in turn to other mutants. The process apparently can be carried through any desired number of steps. As the collection increases in size, it becomes evident that the unlike progeny of tobacco-mosaic virus is capable of causing many diseases that are quite unlike tobacco mosaic. Some of them produce diseases that are so mild that visible symptoms can not be detected, while others give diseases that are lethal. Some of the diseases are caused by viruses of high infectivity, others by viruses of such low infectivity that they are transmitted with great difficulty.

Although possibilities for obtaining new diseases by the isolation of new strains of tobacco-mosaic virus seem almost endless, variability in certain directions is limited. None of the virus mutants have been transmitted to any plant which is immune from tobacco mosaic. Two strains of cucumber mosaic prevalent in England are caused by viruses which serological studies indicate are related to the virus of tobacco mosaic (Bawden and Pirie 1937). Nothing is known regarding the origin of these strains, but it is possible that they are tobacco-mosaic virus mutants which have the ability to infect cucumbers, an ability not possessed by tobacco-mosaic virus. It is of interest to note that these cucumber-mosaic viruses will not infect tobacco. Another case in which closely related strains of viruses have slightly different host ranges was reported in 1932 (Kunkel). The California strain of aster yellows, which in aster plants causes symptoms indistinguishable from those caused by the eastern strain of this disease, affects celery and zinnia plants, which are immune from the latter (Severin 1934). Up to the present time, the new strains of tobacco-mosaic virus have been inoculated into only a few species of plants. Further work may show that some of them can infect plants that are immune from tobacco mosaic. All experimentally isolated strains of tobacco-mosaic virus are alike in that they cause necrotic primary lesions and fail to generalize in *Nicotiana glutinosa.* Holmes (1938) has shown that the failure to generalize is due to a single Mendelian factor which may be transferred to tobacco by hybridization. In leaves of the hybrid plants, ordinary tobacco-mosaic virus causes necrotic lesions similar to those produced by Jensen's No. 14 strain of tobacco-mosaic virus in leaves of unhybridized tobacco plants. Localization was obtained by a change in the genetic constitution of the plant, on the one hand, and by a change in the genetic constitution of the virus on the other. It is interesting to note that up to the present time localization caused by the plant factor has not been broken down, but localization caused by the virus factor has been overcome repeatedly by mutations in Jensen's No. 14 virus.

All of the highly infectious tobacco-mosaic virus mutants and submutants that have been tested show immunological and serological relationships with tobacco-mosaic virus. Nothing whatever is known as to whether any of them could maintain themselves in nature or would be transmitted by insects. Since a number of the mutants cause diseases more severe than tobacco mosaic, care has been taken to prevent their escape from experimental greenhouses.

Results of attempts to obtain information on the frequency of occurrence of mutants in diseased tobacco plants will now be reported. The percentage of unlike progeny obtained from platings made with juice of diseased plants is no measure of frequency of occurrence of mutations, since some and probably all mutants multiply to a limited extent in the host tissues. Many of the bright yellow spots from which yellow mutants have been isolated were rather large and contained considerable quantities of virus. If, as is generally assumed, each spot arises as the result of a single mutation, and if all of the mutations of this type produce spots large enough to be seen, then frequency of occurrence of yellow mutants may be determined by counting the yellow spots. This was done in experimental tests carried out during a period of approximately 15 months.

The chief purpose of the tests was to determine the frequency of occurrence of yellow mutants in mosaic-diseased leaves of Turkish tobacco. Four strains of tobacco-mosaic virus were used in the tests. They were ordinary tobacco-mosaic virus, a derivative of aucuba-mosaic virus, a derivative of Holmes' masked strain of tobacco-mosaic virus, and a derivative of a masked strain of tobacco-mosaic virus obtained from petunia plants. The derivatives were secured by passing each of the three strains in series in tobacco plants until it was replaced by a virus causing green mottling. Each derivative was plated out on leaves of *Nicotiana glutinosa* in order to obtain it in pure culture. The one from aucuba mosaic caused a disease closely resembling tobacco mosaic but differing from it in producing necrotic primary lesions and failing to generalize in *N. sylvestris* Spegaz. and Comes. The other two viruses caused mild green mosaics.

Other purposes of the tests were to determine whether or not yellow mutants would arise with equal frequency in groups of plants affected by the different strains and whether any of the viruses would show seasonal variations as regards production of yellow variants. Seven different tests were made. The plan of each was to inoculate 4 groups of healthy young Turkish tobacco plants growing in deep soil in a greenhouse bench with the four viruses. In each group consisting of 10 plants, 9 were inoculated while the 10th was left uninoculated and served as a control. After the groups had been diseased for a month or longer, 20 mottled leaves on each plant were carefully observed for yellow spots. The spots were counted and the number occurring on each leaf was recorded. The uppermost mature leaf and the 19 leaves immediately below this were the ones examined. The inoculum used in each test was obtained from a single necrotic lesion in a leaf of *Nicotiana glutinosa* on which an appropriate virus plating had been made. A few plants in five of the tests were discarded because they were injured accidentally by supporting stakes, but in no test was any group represented by less than 7 diseased plants. The tests were carried out consecutively. In the first, inoculations and observations were made on November 27 and January 27, respectively; in the second, they were made on February 21 and March 26, respectively; in the third, on April 6 and May 28, respectively; in the fourth, on June 6 and September 2, respectively; in the fifth, on September 9 and October 12, respectively; in the sixth, on November 2 and December 17, respectively; and in the seventh, on December 21 and February 26, respectively. All check plants in all tests remained healthy. The average number of yellow spots in the 20 mottled leaves of the plants infected by each virus in each test is recorded in Table I. The table shows that tobacco-mosaic virus produced slightly more than ½ spot per leaf. In only one test was there an average of less than 1 spot on 2 leaves. In none of the tests did the average reach 1 spot per leaf, and in only one was this value approached. The other three viruses gave larger numbers of spots. That from aucuba mosaic produced an average of slightly more than 3 spots per leaf, while the Holmes masked strain derivative and the petunia masked strain derivative produced an average of more than 1 but less than 2 spots per leaf. The aucuba-mosaic virus derivative produced more spots than any other virus in all of the 7 tests, and in 5 of the 7 it produced more than twice as many as any of the others. It also produced somewhat

TABLE I

RATE OF PRODUCTION OF YELLOW VARIANTS BY STRAINS OF TOBACCO-MOSAIC VIRUS

In tests	1	2	3	4	5	6	7	Average of all tests
Tobacco mosaic virus	13.7	18.8	14.1	13.3	10.5	8.6	10.3	12.7
Aucuba mosaic virus derivative	51.0	64.9	73.5	38.4	34.1	36.0	42.3	48.6
Holmes masked strain tobacco mosaic virus derivative	6.6	18.1	23.8	13.3	17.0	8.4	15.2	14.6
Petunia masked mosaic virus derivative	7.0	28.5	28.0	16.1	22.0	18.7	12.0	18.9

larger spots than the others. The petunia derivative gave more spots than the Holmes derivative in 6 of the tests. All viruses gave more spots in the second and third tests, which were carried out in late winter and early spring, than in tests made in other seasons of the year.

The table shows that the average number of spots per plant did not fall below 6.6 for any virus in any test. The 232 diseased plants grown in the 7 tests bore more than 5000 yellow spots. While some of the spots were small and not very distinct, it is believed that yellow mutants could have been isolated from a large percentage of them. There is little doubt but that other types of mutants were produced in the plants. How large their number may have been is not known.

In conclusion I wish to say that, although studies on tobacco-mosaic virus mutants have not progressed very far, it is clear that tobacco-mosaic virus and its related strains produce a fairly large unlike progeny. It is also clear from work not reported here that several other plant viruses likewise produce unlike progenies. The unlike progenies of all of these viruses are potentially a source of danger, for under favorable conditions they might give rise to new diseases that would be able to survive in nature. They also are of considerable interest from a theoretical standpoint. It is obvious that we must learn to know plant viruses not only by their physical and chemical properties and by the diseases they produce, but also by the progenies they are capable of bringing forth.

REFERENCES CITED

BAWDEN, F. C. and PIRIE, N. W. 1937. The Relationships between Liquid Crystalline Preparations of Cucumber Viruses 3 and 4 and Strains of Tobacco Mosaic Virus. *Brit. J. Exp. Path.*, 18: 275.

BENNETT, C. W. 1930. Further Observations and Experiments on the Curl Disease of Raspberries. *Phytopath.*, 20: 787.

BÖHME, R. W. 1933. Vergleichende Untersuchungen mit Stämmen des "x"- und "y"-virus. *Phytopath. Zeitschr.*, 6: 517.

CARSNER, E. and STAHL, C. F. 1924. The Relation of Chenopodium murale to Curly-top of the Sugar Beet. (Abst.) *Phytopath.*, 14: 57.

CARSNER, E. 1925. Attenuation of the Virus of Sugar Beet Curly-top. *Phytopath.*, 15: 745.

CARSNER, E. and LACKEY, C. F. 1928. Further Studies on Attenuation of the Virus of Sugar Beet Curly-top. (Abst.) *Phytopath.*, 18: 951.

HOGGAN, I. A. 1935. Two Viruses of the Cucumber Mosaic Group on Tobacco. *Ann. Appl. Biol.*, 22: 27.

HOLMES, F. O. 1934. A Masked Strain of Tobacco-mosaic Virus. *Phytopath.*, 24: 845.

———. 1936. Comparison of Derivatives from Distinctive Strains of Tobacco-mosaic Virus. *Phytopath.*, 26: 896.

———. 1938. Inheritance of Resistance to Tobacco-mosaic Disease in Tobacco. *Phytopath.*, 28: 553.

JENSEN, J. H. 1933. Isolation of Yellow-mosaic Viruses from Plants Infected with Tobacco Mosaic. *Phytopath.*, 23: 964.

JOHNSON, J. 1926. The Attenuation of Plant Viruses and the Inactivating Influence of Oxygen. *Science*, 64: 210.

KERLING, L. C. P. 1933. The Anatomy of the "Kroepoek-diseased" Leaf of Nicotiana tabacum and of Zinnia elegans. *Phytopath.*, 23: 175.

Kunkel, L. O. 1932. Celery Yellows of California not Identical with the Aster Yellows of New York. *Contrib. Boyce Thompson Inst.*, 4: 405.

Lackey, C. F. 1929. Attenuation of Curly-top Virus by Resistant Sugar Beets which are Symptomless Carriers. *Phytopath.*, 19: 975.

————. 1929a. Further Studies of the Modification of Sugar-beet Curly-top Virus by Its Various Hosts. (Abst.) *Phytopath.*, 19: 1141.

McKinney, H. H. 1926. Virus Mixtures That May Not Be Detected in Young Tobacco Plants. *Phytopath.*, 16: 893.

————. 1929. Mosaic Diseases in the Canary Islands, West Africa, and Gibraltar. *J. Agr. Res.*, 39: 557.

Price, W. C. 1934. Isolation and Study of Some Yellow Strains of Cucumber Mosaic. *Phytopath.*, 24: 743.

Salaman, R. N. 1933. Protective Inoculation against a Plant Virus. *Nature*, 131: 468.

Severin, H. H. P. 1934. Experiments with Aster-yellows Virus from Several States. *Hilgardia*, 8: 305.

Storey, H. H. and McClean, A. P. D. 1930. The Transmission of Streak Disease between Maize, Sugar Cane and Wild Grasses. *Ann. Appl. Biol.*, 17: 691.

Storey, H. H. 1931. The Bearing of Insect-vectors on the Differentiation and Classification of Plant Viruses. *II^e Congr. Intern. Path. Comp., Vol. 2, Comp. rend. et Communic.*, pp. 471–479.

Thung, T. H. 1932. De krul- en kroepoek-ziekten van tabak en de oorzaaken van hare verbreiding. *Proefsta. Vorstenl. Tabak Meded.* 72. 54 pp.

PROBLEMS IN THE VARIATION OF PATHOGENIC BACTERIA

By G. B. REED

QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA

From the early days of Pasteur and Koch down to the last decade and a half, pathogenic bacteriology was dominated by the dogma of *monomorphism.* With few exceptions, the earlier studies were more concerned with establishing the fixity of species than with variation. But during the last 15 to 18 years the literature of the subject has been flooded with evidences of variability.

It has become increasingly apparent that pathogenic species of bacteria may pass through thousands of generations under laboratory conditions or in the animal body without detectable change in their characteristics. At the same time, it is equally evident that most, and probably all, pathogenic species at times undergo variation. The fact that bacteria do vary has repercussions in almost every field of the study of infectious diseases. I should like to call attention to problems connected with variations in antigenic composition and virulence and to suggest some hypothetical considerations concerning the causes of variations.

Variations in Antigenic Composition

From the early work, especially that of Arkwright (1920), it has been apparent that certain variations are generally correlated. The most frequently observed variation, the Smooth to Rough (S to R) colony form, is not merely a change in the structure of the colony; there is usually associated a change in virulence, in antigenic composition and other characteristics, such as cell morphology, sensitivity to bacteriophage, and hydrophobe or hydrophile properties of the cell. The variation is generally discontinuous and the variants are frequently highly stable. In many species the S to R change occurs readily, but reversion or R to S occurs only rarely. The significance of this type of variation is not apparent, but it seems safe to assume that it represents a relatively major change in the genetic makeup of the organism.

The pathological importance of the change is primarily due to variation in the antigenic composition which appears to be a fundamental consideration in virulence as well as in antibody stimulating and reacting mechanism. The S to R variation ordinarily involves a loss in the heat-stable somatic antigen that characterizes the surface of the normal virulent type. This loss may further result in the uncovering of somatic antigens which then dominate the variant type. The somatic antigen, probably most frequently concerned in this loss variation, we know, from the initial work of Heidelberger and Avery, is a polysaccharide. In chemically pure form the polysaccharides are haptens or partial antigens —they react with antibodies but do not stimulate antibody formation. In the cell, however, they act as antigens. In the case of the *Pneumococci,* the polysaccharide is contained in the capsule. In the course of the S to R variation the capsule and the specific polysaccharide antigen are lost. There remains, however, in the R organisms an antigenic protein component common to all types of *Pneumococci.* It has been shown that a similar series of changes accompany S to R variation in several, possibly all, pathogenic species. This loss of surface antigen means that the variant lacks antigenic specificity and lacks the ability to stimulate the formation of specific antibodies in animals. It has not been possible to put all antigenic differences between bacterial species or variants into chemical terms or to interpret antigenic variation in this precise manner, but the fact that certain major changes can be so interpreted tends to clarify the situation.

Knowledge gained from a study of variation in the antigenicity of bacteria, during the last dozen years, has resulted in many modifications in methods of immunization and diagnosis by immunological means. We now know that in the preparation of vaccines for immunization against typhoid, paratyphoid, and other infections of this group, consideration must be given to the complex antigenic composition of the organisms and the ease with which variation in these components occurs under laboratory conditions. Similar problems are met with to a greater or lesser degree in the preparation of vaccines from all types of pathogenic bacteria. Similarly, in the preparation of antisera, as against *Pneumococci* and *Meningococci,* possible variation in antigenic composition is a fundamental problem. In such a simple procedure as the Widal and other agglutination reactions used in the identification of antibodies for diagnosis of infections, due regard must be given to the antigenic structure and possible variation of the organisms used as antigens. Such problems appear in almost every phase of immunology.

It will be recalled that the French group —Calmette *et al.*—obtained from an old culture of tubercle bacilli a strain, BCG, of exceedingly low virulence, a type capable of producing only localized lesions which are fairly rapidly absorbed. It was shown by Petroff (1927), Begbie (1930), Sasano and Medlar (1931), Seiffert (1932), and ourselves (1934) that this organism is probably an R variant of the normal tubercle bacillus. It has been found that the strain generally is highly stable like most R forms. In the hands of a few observers, sufficient variation has, however, occurred to permit recovery of the fully virulent or normal S form (Reed, Orr, and Rice, 1934).

The French group and others have long claimed that the introduction of BCG cultures into infants results in a considerable degree of immunity against ordinary tuberculous infection. This vitally important problem has been seriously questioned on statistical grounds. We have approached it differently by examining the antigenic structure of the BCG organisms in contrast to normal tubercle bacilli.

In earlier papers (Rice 1931; Rice and Reed 1932) it was shown that, if rabbits are immunized with whole cultures of normal S forms and other rabbits with R variants of mammalian tubercle bacilli, a distinction can be made in the antibody content of the two lots of animal sera. The examination procedure was an indirect one involving complement fixation with antigens prepared from S and R types of organisms. The anti-S immune serum fixed, on the average, twice as much complement with the S antigen as was fixed with R antigen. The anti-R serum fixed, on the average, the same amount of complement with both S and R antigens. These observations were interpreted to mean that the S organisms contain an S-specific and a species antigen, and that the R organisms lack the S-specific antigen but contain the species antigen. Correspondingly, the anti-S serum contains the two antibodies; the anti-R serum, the species antibody, while it lacks the S-specific antibody.

The same examination procedure was followed with the ordinary BCG cultures and the S type recovered from them (Reed, Orr, and Rice 1934). Rabbits were immunized with the two types and the antisera tested against antigens prepared from S and R type mammalian tubercle bacilli. The results are summarized in Table I. It is apparent from the column on the left that antisera prepared from several cultures of BCG contain antibodies which fix complement in approximately equal amounts in the presence of S and R antigen. The antisera prepared from S variants of the BCG (which are virulent), as indicated on the right of Table I, fixed approximately twice as much complement in the presence of the S antigen as in the presence of the R antigen. The simplest explanation of the observations is that the BCG organism, like other R tubercle bacilli, lacks an antigenic component which characterizes normal virulent tubercle bacilli. Lacking this antigen, the corresponding antibody is lacking in the serum of BCG immunized animals.

TABLE I

A Comparison of the Reactivity of Typical S and R Mammalian Tubercle Bacillus Antigens with the Serum of Rabbits Immunized with Ordinary BCG–R Organisms and with Dissociated S Organisms as Measured in Cubic Centimeters of Complement Fixed Specifically at Fifty Per Cent Hemolysis

Antiserum	Antigen	Specific fixation	Antiserum	Antigen	Specific fixation
BCG–R Montreal (1)	S R	.0041 .0035	BCG–S Trudeau	S R	.0064 .0030
BCG–R Montreal (2)	S R	.0080 .0074	BCG–S Alberta (1)	S R	.0032 .0014
BCG–R Montreal (3)	S R	.0147 .0139	BCG–S Alberta (2)	S R	.0143 .0087
BCG–R Montreal (4)	S R	.0110 .0101	BCG–S Alberta (3)	S R	.0143 .0066
BCG–R Montreal (5)	S R	.0073 .0069	BCG–S Ottawa (1)	S R	.0057 .0028
BCG–R Paris	S R	.0090 .0080	BCG–S Alberta (4)	S R	.0150 .0087
BCG–S & R Alberta (5)	S R	.0100 .0082			

It seems probable that the BCG organism has arisen as a variant from "normal" tubercle bacilli and that one feature of the variation was a loss of a specific antigenic substance. This adds support to the doubt raised from the statistical analysis based upon the use of the material as an immunizing agent in man and cattle. It is in line, too, with general findings with other species of pathogenic bacteria, such as the *Pneumococci,* members of the typhoid coli group, that the S to R type of variation is accompanied by a loss of a specific antigen which renders the variant ineffective as an immunizing agent.

Variation in Virulence

A long recognized type of variation is change in virulence or change in ability to produce toxins. This change occurs in many species under laboratory conditions, and we know that avirulent or atoxogenic variants may frequently be isolated from man (Wadsworth and Sickles 1927; Okell 1929; Gunn and Griffith 1928). Variation in the animal body is probably related to the interaction of antibodies with the surface antigens of the organisms.

An attractive explanation of the rise and fall of epidemics has been based, with some degree of experimental support, upon these findings. The extent to which host immunity and bacterial variation contribute to the course of epidemics has never been determined, but there seems to be ample evidence from both the study of epidemic disease in man and experimental herd infections in animals that variation in the causal bacteria is at least a factor of importance, even though it be not a simple and obvious relationship.

In a long series of studies of herd epidemics in mice, Webster and colleagues (1930, 1933) have shown that different strains of the same bacterium recovered at intervals during the course of one epidemic may possess an approximately equal virulence. This they regularly found to be the case. They, therefore, conclude that changes in virulence play no part in the fluctuations in mortality frequently observed in a long continued epidemic. They have, however, reported different degrees of virulence in the same bacterial species recovered in different epidemics (1930). At the same time, they demonstrated that in one species of

Pneumococcus (Webster and Clow 1933), a strain of high virulence, as judged by intraperitoneal and intranasal infection, might as a result of nose-to-nose passage, lose its intranasal virulence without affecting its intraperitoneal virulence.

On the other hand, Topley, Greenwood, and associates (1928) have considered a second factor. They find that virulence, as measured by direct inoculation, and the power to induce severe epidemics by contact infection are not always correlated. Recent experiments with a species of *Pasteurella* (Greenwood *et al.* 1936) seem to afford proof that changes in infectivity may occur during the epidemic spread of a bacterial parasite. They, therefore, suggest that an "epidemic strain" is characterized by two attributes, high virulence and high infectivity. Loss of either through variation may result in loss of ability to cause an epidemic. It seems safe to conclude from these herd studies that variation in the invading bacteria may constitute a major factor in the spread of human infections. There is also much supporting evidence to be found in a study of human infections.

THEORETICAL CONSIDERATIONS

The similarity of the variation pattern in many species of bacteria and especially the variation of correlated characteristics, as just noted, have contributed largely to the theory of cyclic change in the life history of the bacteria. But variation by no means always occurs simultaneously in several characteristics. In a recently published study (Reed 1937) of the saprophytic species, *Serratia marcescens* Bizio, it was shown that variation may occur independently in several characteristics.

In this species, as is generally the case, colony structure, cell form, and specific somatic agglutinogen are always associated: S colonies are composed of short rods and coccoid cells containing specific somatic antigen, while the R colonies are composed of long rods and filaments without the specific somatic antigen. Where variation occurs from S to R or in the reverse direction, the associated characteristics vary synchronously. This may arise from an inseparable linkage of the inheritance factors or these three characteristics may result from the transmission of a single gene. What is measured as somatic antigen may, for instance, determine the cell form and cell form may determine the colony topography.

Several other characteristics exhibited independent variation. Capsulated cells with the resulting mucoid structure of colonies occur in both S and R forms. Variation may proceed in the direction of either gain or loss in capsulation, and this variation may occur irrespective of variation in S or R colony form. It is evident, therefore, that the factor for the inheritance of mucoidness or its absence is independent of the factor or factors for colony structure. Again, variation in pigmentation from red to orange-red or white, or from orange-red to white, occurs regardless of variation in colony structure or capsulation of the cells. Color factors must, therefore, be inherited independently of factors for colony structure or for capsulation.

Independent variation in three sets of characteristics does not appear to be consistent with any theory of cyclic change in the organisms. It seems more likely that, in these cases at least, the inheritance of individual characteristics has been subject to some irregularity. It is more in line with current genetical opinion to assume that this is the result of change in individual genes or "gene mutation." A study of *Staphylococci* now in progress adds additional evidence in support of this hypothesis.

There are, however, certain well known variations in pathogenic bacteria which possibly permit a simplification of the gene hypothesis, a simplification which may be applicable to all inheritance and variation in the bacteria, and perhaps in a wider field.

Since the work of Neufeld in 1909, we have been familiar with the existence of antigenically different types of *Pneumococci.* Three types and a heterogeneous group were first distinguished, and quite recently the unclassified group has been differentiated into some 27 additional types. There is ample evidence that the antigenic difference

TABLE II

Characteristics of the Type-Specific Polysaccharides of Pneumococci
(After Avery and Heidelberger)

Type	Optical rotation	Per cent nitrogen	Substances obtained on hydrolysis	Dilution giving specific precipitation
I	300°	5	Galacturic acid and amino-sugar derivatives	1: 6,000,000
II	74°	0	Glucose	1: 5,000,000
III	– 33°	0	Glucose and glucoronic acid	1: 6,000,000

between at least the original three types depends upon chemical differences in the polysaccharide, which is the principal component of the capsule surrounding the organism, as indicated in Table II.

As just noted in another connection, variation frequently occurs in the strain of this species in which the capsule and type-specific polysaccharide is lost. Such variants, therefore, do not belong to a specific type; they have lost their type characteristic. It was shown by Griffith (1928) and recently confirmed particularly by Dawson and Sia (1931) that, if a non-capsulated, non-type-specific variant arising from type I is grown in a menstruum including type II polysaccharide, reversion to a capsulated type-specific form may occur. And, very significantly, the new form will be not type I from which the non-capsulated strain arose but type II, the type of the polysaccharide present in the menstruum in which the variation occurred. Once this new type II has arisen, it continues to breed as a characteristic type II; that is, the newly gained ability to synthesize the type II polysaccharide is passed on from generation to generation.

The fact that the type-specific polysaccharide is taken up from the environment and continues to be formed in subsequent generations suggests that it may be a self-propagating substance like Stanley's virus protein. If so, the type-specific inheritance may be regarded simply as a cutting off, in the fission division, of a portion of the substance sufficient to permit the propagation of more similar substance in the developing daughter cell. A loss variation is then simply a cell division in which the polysaccharide fails to divide. If this is true of specific polysaccharide, possibly there are in the cell other self-propagating substances. Such a simple hypothetical mechanism probably makes it unnecessary to postulate genes, although it is conceivable that the genes are just such substances.

References Cited

Arkwright, J. A. 1920. Variation in Bacteria in Relation to Agglutination by Salts and by Specific Sera. *J. Path. & Bact.*, 23: 358.

Begbie, R. S. 1930. Microbic Dissociation, with Special Reference to Certain Acid-fast Bacilli. *Edinburgh Med. J.*, 37: 187.

Dawson, M. H. and Sia, R. H. P. 1931. In vitro Transformation of Pneumococcal Types. I. A Technique for Inducing Transformation of Pneumococcal Types in vitro. *J. Exp. Med.*, 54: 681.

Greenwood, M., Hill, A. B., Topley, W. W. C. and Wilson, J. 1936. Experimental Epidemiology. *Med. Res. Council Sp. Rept.*, no. 209.

Griffith, F. 1928. Significance of Pneumococcal Types. *J. Hyg.*, 27: 113.

Gunn, W. and Griffith, F. 1928. Bacteriological and Clinical Study of 100 Cases of Scarlet Fever. *J. Hyg.*, 28: 250.

Okell, C. C. Relationship of Virulent to Avirulent Diphtheria Bacilli. *J. Hyg.*, 29: 309.

Petroff, S. A. 1927. Microbe Dissociation: The Tubercle Bacillus. *Proc. Soc. Exp. Biol. & Med.*, 24: 632, 956.

Reed, G. B., Orr, J. H. and Rice, C. E. 1934. Studies in Variability of Tubercle Bacilli; BCG. *Canad. J. Res.*, 11: 362.

Reed, G. B. 1937. Independent Variation of Several Characteristics in S. marcescens. *J. Bact.*, 34: 255.

Rice, C. E. 1931. Studies in the Variability of Tubercle Bacilli. IV. Antigenic Properties of S and R Cultures. *Canad. J. Res.*, 5: 375.

Rice, C. E. and Reed, G. B. 1932. Studies in the Variability of Tubercle Bacilli. VII. Antigenic Activity of "S" and "R" Cultures as Measured by Complement-fixation. *J. Immunol.*, 23: 385.

SASANO, K. T. and MEDLAR, E. M. 1931. Studies on the Calmette-Guérin Strain of the Tubercle Bacillus (BCG). I. The Effect in vitro of Environment on the Virulence of BCG. *Amer. Rev. Tuberc.*, 23: 215.

SEIFFERT. 1932. Dissoziationsversuche mit BCG. *Klin. Wochenschr.*, 11: 181.

TOPLEY, W. W. C., GREENWOOD, M., WILSON, J. and NEWBOLD, E. M. 1928. Epidemic Potency of Strains of Bact. aertrycke of Varying Virulence; Report to Medical Research Council. *J. Hyg.*, 27: 396.

WADSWORTH, A. B. and SICKLES, G. M. 1927. A Study of Pneumococci Isolated from Horses Undergoing Pneumococcus Immunization. *J. Exp. Med.*, 45: 787.

WEBSTER, L. T. 1930. The Role of Microbic Virulence, Dosage and Host Resistance in Determining the Spread of Bacterial Infections among Mice. III. B. enteritidis Infection. *J. Exp. Med.*, 52: 931.

WEBSTER, L. T. and CLOW, A. D. 1933. Intranasal Virulence of Pneumococci for Mice. *J. Exp. Med.*, 58: 465.

INFLUENCE OF HEREDITY AND ENVIRONMENT ON PATHOGENICITY IN BACTERIA

By H. KONST

ANIMAL DISEASES RESEARCH INSTITUTE, DEPARTMENT OF AGRICULTURE, HULL, QUEBEC, CANADA

Bacteria are not stable with respect to either morphological or physiological characteristics, but, instead, give rise to innumerable variants. Extensive studies have shown that variants better adapted to the existing environmental conditions will eventually replace parent types. Alterations in the environmental conditions may thus seemingly cause changes in the characteristics of the bacteria. In culture, replacement of a pathogenic parent type by an attenuated variant will cause loss of virulence. In nature, variant strains may spread rapidly and produce epidemics or epizootics. In some instances the variant may be sufficiently distinct from its parent to be considered as a new species. It would be desirable to study bacterial variations in culture, since such study might lead to a better understanding of the interrelationships of bacterial species.

It is the purpose of this discussion to review some of the information available on variation of pathogenic bacteria and to compare, insofar as it is possible, the variations observed in nature with those that may be induced in culture. Such widely different bacteria as *Bacillus anthracis* Koch, the anthrax bacillus; *Erysipelothrix rhusiopathiae* (Mig.) Holl., the causal agent of swine erysipelas; *Pasteurella aviseptica* (Kitt.), the fowl cholera bacterium; *Actinomyces bovis* Harz, the cause of actinomycosis in cattle; *Mycobacterium tuberculosis* (Koch) L. and N.; and *M. paratuberculosis* (Johne) Bergey *et al.* have been selected for discussion. It is believed that the observations on these bacteria give a fair cross section of the evidence accumulated on variations in virulence of bacteria in general.

The Anthrax Bacillus

The anthrax bacillus was not only the first bacterium definitely shown to cause disease but was also one of the first observed to produce avirulent strains. Pasteur (see Hutyra and Marek 1922 and Eurich and Hewlett 1930) discovered in 1881 that virulent strains cultured at 42° to 43° C frequently produce attenuated, asporogenous variants. Preisz (1904) extended these observations in 1904 and showed that numerous strains of the bacillus exist in man and animals. He found that 12 freshly isolated cultures differed considerably in their morphology, virulence, and cultural characteristics. Müller (see Hutyra and Marek 1922, p. 5) isolated the asporogenous strain from cow's blood and found that it did not change in culture.

While Preisz was attempting to improve on the Pasteur vaccine for anthrax, he (1911) found that variants could be isolated from cultures by the dilution plate method. Some of the cultures completely lost their virulence in the course of time. The loss was correlated with a change from a Rough to a Smooth type. He concluded, therefore, that the attenuation of cultures at 42.5° C was brought about by an alteration in the capsule-forming ability of the bacillus. Further evidence in support of this conclusion was offered in 1937 by Hegyeli, who found that when the attenuated strains were restored to virulence they simultaneously reverted to the Rough type. In order to bring about this change, he used different media, such as ordinary bouillon supplemented with spleen extracts, normal swine serum, horse serum, and egg-white. The best results were obtained by cultivating cultures in fresh egg-white bouillon medium. Cultures of the anthrax bacillus may also become attenuated when treated with potassium bichromate or with phenol, or when exposed to direct sunlight or to an increased atmospheric pressure (Chamberland and Roux 1883; Chauveau—see Hadley 1927, p. 165; Arloing 1885).

Very little is known about the biochemical characteristics of the anthrax variants. Buzna (1925) has found differences in the sugar-fermentative power between vaccine strains, avirulent asporogenous strains, and fully virulent strains. Although the examinations were not made on isolated, true-breeding variants, the results nevertheless suggest a parallelism between morphological and biochemical properties of the predominant variants in the cultures.

Swine Erysipelas Bacillus

Swine erysipelas occurs as an acute, septicaemic disease and as a chronic, benign disease mainly affecting the joints. In Europe, it occurs predominantly in the acute form both enzootically and epizootically. In North America, this form has not occurred until recently. A septicaemic type of erysipelas was observed for the first time in South Dakota by Fosterman (1932) in 1930. From this relatively isolated region, the new form has spread to several other states and is becoming important economically in the midwestern section of the United States (Breed 1938). It was observed in Saskatchewan, Canada, in 1933 by Fulton who emphasized, however, the prevalence of the chronic articular type. He has secured from this prevalent type attenuated cultures that produce only articular lesions.

A fairly high percentage of the hogs killed in slaughter houses have arthritis, which supposedly results from chronic lesions of erysipelas. In preliminary studies on these arthritis cases, Heath (personal communication) and McIntosh and Mackie (personal communication) failed to find the swine erysipelas bacillus in some of the animals examined. They did recover the bacillus from certain of the lesions but found very few of the cultures to be highly pathogenic for mice. Filamentous forms were always found among the short, Gram-positive rods that are characteristic of the species.

The foregoing evidence indicates that there are at least two strains of the swine erysipelas bacillus, the highly virulent strain prevalent in Europe and the weakly virulent strain prevalent in North America. Although the highly virulent strain recently observed in the United States may have been imported from Europe, there is no direct evidence of this. The evidence indicates, on the other hand, that the highly virulent strain developed from the weakly virulent one. In this connection, it is interesting to note that *Erysipelothrix murisepticae* (Flügge) Bergey *et al.,* first found by Koch in putrid blood, is generally considered to be an attenuated strain of *E. rhusiopathiae.* The two bacteria differ in their virulence for swine but are identical in their morphological, serological, and immunological characteristics. Both are equally virulent for small laboratory animals (Hutyra and Marek 1922; Edwards 1931).

The erysipelas bacillus exhibits variability not only in nature but also in culture. The first evidence for variation *in vitro* was obtained by Pasteur while developing his well known methods of immunization. He and Thuillier observed that the bacillus became attenuated for swine during incubation in rabbits that are relatively resistant. These attenuated strains did not change in virulence when cultured on bouillon at body temperature. Such cultures were used as the vaccine No. I of Pasteur. Passage of the strains through pigeons that are highly susceptible increases their pathogenicity for swine; such strains are used in making vaccine No. II (Hutyra and Marek 1922; Edwards 1931; Topley and Wilson 1929).

Long thread-like forms of the bacillus are frequently found in the lesions of *endocarditis verrucosa* which is observed mainly in European countries in the chronic form of erysipelas. Redlich (1932) separated cultures from such sources into Rough and Smooth colonies and found that the Rough ones were composed of filamentous-type bacteria, the Smooth ones of rod-like bacteria. The Rough forms were the more attenuated of the two. Hegyeli (1931) also found the development of filamentous forms in broth cultures to be correlated with attenuation. On the other hand, Darzine (1926) and Panisset and Kolda (1924) found that appearance of thread-like organisms in bile bouillon apparently was not followed by attenuation.

Fowl Cholera Bacterium

The close relationship of the various members of the *Pasteurella* group has made it impossible to differentiate sharply between them. According to Schütze (1929) of the Lister Institute, "The Pasteurellas should be regarded as a collection of strains fundamentally identical, though capable of adaptation in respect to one or the other animal host." One member of this rather general group is *P. aviseptica,* the causal agent of fowl cholera. This bacterium is of special historical and scientific interest because it was the micro-organism with which Pasteur (see Schütze 1929) first demonstrated the feasibility of using artificially attenuated cultures for prophylactic vaccination.

Under natural conditions *Pasteurella aviseptica* varies considerably in its virulence for hens. Most strains are so highly virulent that a few cells constitute a lethal dose (Stang 1901; Bujwid 1919; Hadley 1912). Cultures have been isolated, however, by Mack and Records (1916) and Hadley (1912, 1913) which were not lethal even when used in large doses. They caused emaciation and a mild chronic arthritis. Most investigators regard the bacterium as an obligate parasite even though some American workers (Mack and Records 1916 and Gomez 1926) maintain that it may occur, in an avirulent state, as a saprophyte in soil or stagnant water.

Since Pasteur's attenuated strains, obtained by prolonged exposure of broth cultures to air, did not yield satisfactory results in practical application, several attempts have been made to develop better vaccines. Hadley (1913, 1915) obtained a weakly virulent strain that became further attenuated after being retained in culture for over three years. This attenuated strain was used to partially immunize rabbits so that they could survive injection with small doses of a highly virulent strain. This method of vaccination resulted in a high degree of immunity. The method later proved successful with fowl. From aged cultures of *Pasteurella aviseptica,* Manninger (1919) isolated a completely avirulent, true-breeding variant with distinctive morphological, cultural, and biological properties. When used for vaccination, this variant conferred an active immunity of from 2 to 4 months' duration. Manninger ascribes the attenuation in his cultures to the influence of accumulating metabolic products that favor the development and reproduction of avirulent individuals in the bacterial population. Hadley (1927) pointed out that Manninger apparently obtained a Rough variant of *P. aviseptica* which had become predominant in his cultures.

Cattle Actinomycosis Organism

The organisms classified as *Actinomycetes* vary greatly in their morphological and physiological properties. Many of them occur in the soil as saprophytes; some of them are capable of causing disease in plants, while others cause disease in man and animals. The first of these to be studied was *Actinomyces bovis* which Bollinger, in 1877, recognized as the cause of a pathological condition of the jaw bone in cows. The botanist Harz identified the granular bodies found in the lesions as being of a fungous nature and established the genus *Actinomyces* Harz, for organisms of this type. This generic name is now generally accepted for all of the ray-fungi which are characterized by the radial arrangement of club-shaped threads at the periphery of mycelium-like colonies.

There are both aerobic and anaerobic species of *Actinomyces.* Both types cause lesions in man and animals, the most important anaerobic species probably being *Actinomyces bovis,* type Wolff-Israël. One of the aerobic species causes the so-called Madura foot in man and a second is responsible for Farcy in cattle. These two species are either not acid-fast or are weakly so, but others, such as *A. asteroides* Epp. and *A. gypsoides,* from pulmonary and abdominal infections in man, are acid-fast. All species are Gram-positive.

For some time it was believed that the saprophytic species of *Actinomyces* found in soil and on grass became pathogenic after entering the animal body. This belief was based on Bostroem's findings in 1890. However, more recent work by Wolff-Israël, Wright (1904–5), Colebrook (1921), and many others has shown that the parasitic, anaerobic type described by Wolff-Israël is, for the most part, the cause of actinomycosis in man and cattle (Bulloch *et al.* 1931; Sedlmeier 1936; Topley and Wilson 1929).

Foulerton (1910, 1912) advocated that the acid-fast bacteria of the *Mycobacteriaceae* be included in the *Actinomycetaceae* because of the close relation between the two families. Claypole (1913) has since shown by complement fixation tests that *Mycobacterium tuberculosis* is similar to some species of *Actinomyces.* Mitchell (personal communication) has also shown a serological reaction between the tubercle bacillus and the acid-fast species, *A. gypsoides* and *A. asteroides.* The tubercle bacillus did not react serologically with the non-acid-fast *A. bovis.* There was, however, a cross complement fixation reaction between *A. bovis* and the acid-fast species. Mitchell thought that the antigenic relationships observed in this series might be explained on the basis of an evolutionary development in the different species.

Tubercle Bacillus

Of the numerous members in the group of *Mycobacteria,* we are here concerned only with *Mycobacterium tuberculosis,* especially with its bovine type, and with *M. paratuberculosis.* It is generally accepted that the various members of the species *M. tuberculosis* are phylogenetically of identical origin. There is no unanimous opinion regarding the specificity of the types known at present, but it may be safely stated that

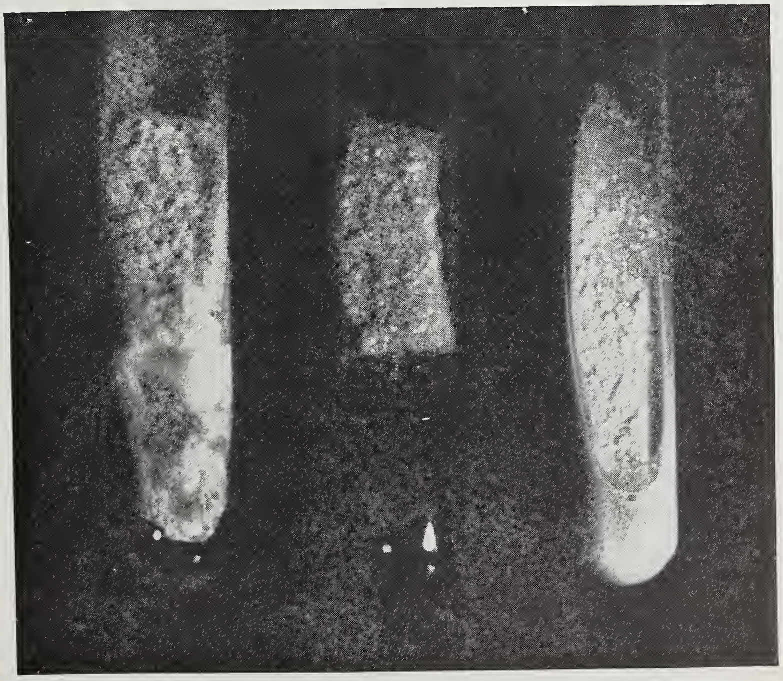

Fig. 1. Growth of a virulent strain of *Mycobacterium tuberculosis* on ordinary glycerinated potato broth medium, Calmette's glycerinated ox bile-potato medium, and on Petroff's gentian violet egg medium.

most bacteriologists now regard the various members as being distinctly different (Gotschlich 1929).

Atypical strains occur very rarely in nature. Such strains have, however, been isolated by members of the Royal Commission (1911) from lymph glands of man; by Rabinowitsch (1906) from infected monkeys; and by Griffith (1924) from lupus. From a crow, Mitchell and Duthie (1929) isolated an atypical strain that was pathogenic for chickens, guinea pigs, and rabbits. The strain was less virulent for rabbits than for guinea pigs and chickens. It remained virulent for guinea pigs after passage through chickens and was fully virulent for chickens after incubation in guinea pigs.

The claim has been made that some strains of the tubercle bacillus may be modified by artificial means, such as passage through a heterologous host, but many of the experiments will not stand critical examination. In the final analysis, such experiments are open to question unless there is some assurance that the host did not develop spontaneous tuberculosis and that the cultures used were free of variant strains.

There is much better evidence of changes of *Mycobacterium tuberculosis* in culture. Cultures incubated under special conditions may change either in their morphological and cultural characteristics or in their pathogenicity and other biological properties (Kirchner 1931; Lange 1932). For example, Calmette and Guérin (1924) found that a highly virulent bovine strain became fully attenuated after 230 passages on a glycerinated ox bile-potato medium over a period of 13 years. They claimed that the attenuated strain, designated as BCG, was completely innocuous for all small laboratory animals, cows, and man, yet had a pronounced immunizing property. Although the opinion regarding the latter

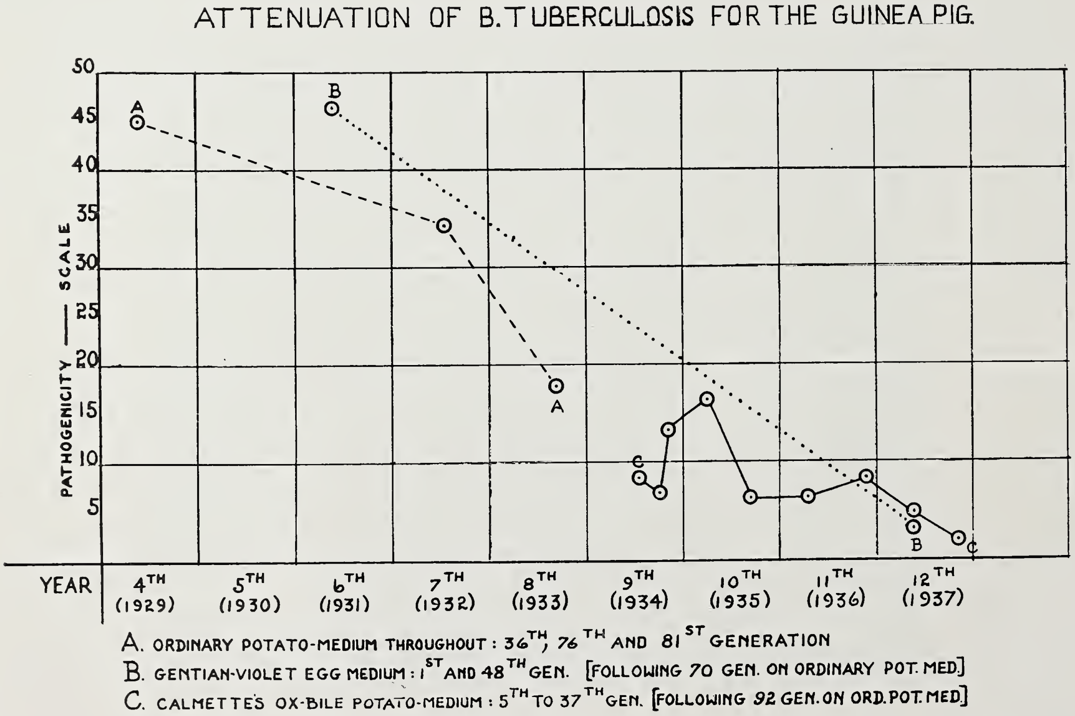

Fig. 2. Pathogenicity of *Mycobacterium tuberculosis* for guinea pigs after monthly serial transfers on ordinary potato medium (A), gentian violet egg medium (B), and Calmette's ox bile-potato medium (C).

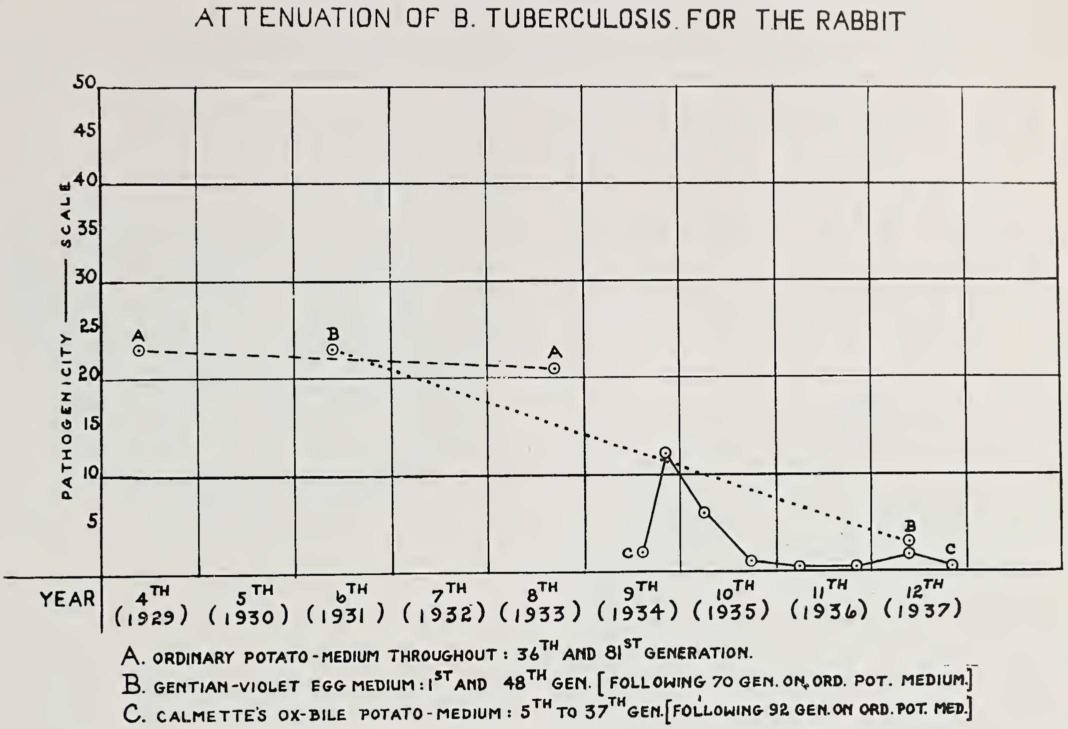

FIG. 3. Pathogenicity of *Mycobacterium tuberculosis* for rabbits after monthly serial transfers on ordinary potato medium (A), gentian violet egg medium (B), and Calmette's ox bile-potato medium (C).

claim is still divided, BCG cultures are used in many countries for prophylactic vaccination of children on a large scale. In 1933, Calmette, Guérin, and Nègre reported the complete attenuation of a human strain after 418 successive transfers during a period of 22 years on ox bile medium. It was necessary, however, to acclimatize this strain to ox bile by carrying it for over one year on a human bile-potato medium.

Further light has been thrown on the circumstances governing variability of the tubercle bacillus *in vitro* by studying the phenomenon of microbic dissociation. Petroff and his coworkers have the credit for initiating such studies. They observed that this bacillus, like many other micro-organisms, may produce Rough, Smooth, and intermediate types of variants. These observations facilitated an understanding of the fundamentals underlying variability. The characteristics of a culture could be changed very easily by creating conditions which would favor one strain more than others in the culture. Development of the attenuated BCG culture on potato-ox bile medium is considered to be due to the selective growth of a Rough type of variant on this medium. For example, the experiments of Petroff, Branch and Steenken (1929) and of Reed, Orr, and Rice (1934) have shown that on this medium there is an increase in the Rough variants and a decrease in the Smooth type. By altering the cultural conditions, they were able to reverse the trend so that the Smooth type became predominant. The Smooth types obtained from BCG cultures proved to be virulent.

Watson and his coworkers (1928, 1933) obtained similar results by inoculating guinea pigs subcutaneously or intraperitoneally with suspensions of three strains of BCG cultures obtained from the Pasteur Institute, Lille and Paris. Four of the cultures reisolated from these guinea pigs were virulent and of the usual Smooth bovine type. One of the virulent substrains, obtained from an animal inoculated with the Pasteur Institute strain No. BCG 80, showed a marked decrease in virulence after serial transfer on glycerinated potato broth medium for several years. This substrain was obtained by the author and transferred to Calmette's ox bile-potato medium in order to attenuate it completely. Serial subcultures were made each month on this medium, on ordinary potato medium, and on Petroff's egg medium. The growth on ox bile was, from the beginning, gray-green, smooth, and slightly glistening, with superimposed hemispherical mounds (Fig. 1, center tube). A duller, dry type of growth was produced on other media (Fig. 1, first and third tubes).

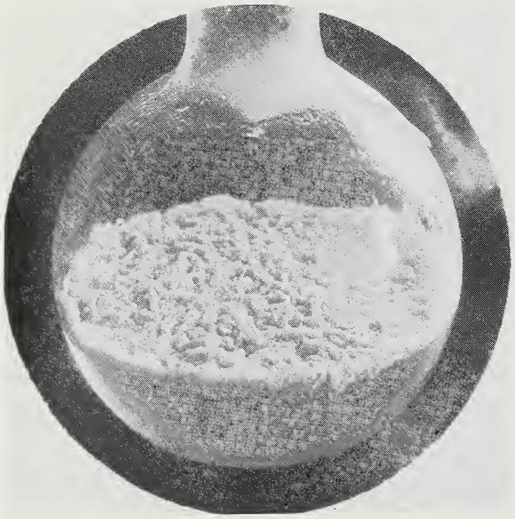

Fig. 4. Type of growth produced by Johne's bacillus, *Mycobacterium paratuberculosis*, on Long's synthetic medium adjusted to pH 5.5 to 5.8 devoid of *M. phlei* extract.

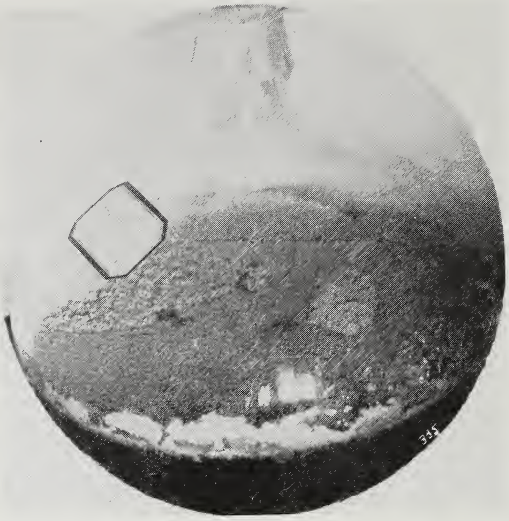

Fig. 5. Strain No. 1 before changing.

The culture on ox bile medium was tested for pathogenicity on nine different occasions between the fifth and thirty-seventh transfers. The tests were usually made at intervals of 4 to 5 months. In each test, 4 guinea pigs were inoculated intraperitoneally with 1, 1.5, 2, and 2.5 mg of culture, respectively, and a rabbit intravenously with 1 mg. The degree of virulence was expressed according to the scale used in our laboratory for assaying tuberculous lesions. A maximum infection is represented by 100, but even the most virulent cultures seldom produce more than 75 to 85 per cent involvement. The culture used in

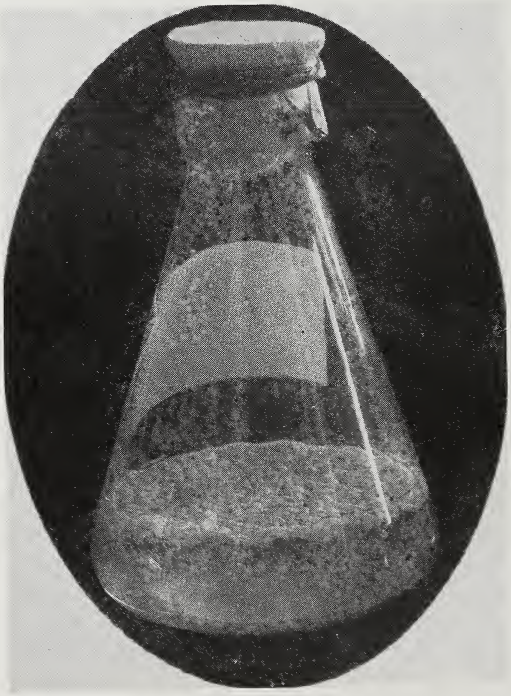

Fig. 6. The smooth type of growth produced by Johne's bacillus, strain No. 1, after a period of culture on Long's synthetic medium at pH 6 to 6.4.

these tests never produced more than 16 per cent, so it may be considered as fairly well attenuated and capable of producing only local lesions. The lesions produced during the last two years of the test were smaller than those produced at the beginning (as is shown in Fig. 2). The culture was so attenuated that it was comparable to the true BCG strain from which its virulent parent type was derived. This attenuation came about after 8½ years on an ordinary medium and 3½ years on Calmette's ox bile medium. Fig. 2 summarizes the data on this culture.

The subculture maintained on Petroff's egg medium during this same period was just as attenuated as that on ox bile, as is also shown in Fig. 2. Both cultures were able to produce lesions, however, when exceedingly heavy doses (up to 7 mg) were used. Results from these heavy inoculations indicated that the culture on ox bile was the more attenuated of the two. Fig. 3 shows that tests with rabbits gave results similar to those with guinea pigs. The cultures on ox bile and egg media were completely avirulent, even when doses of 6 mg were injected intravenously. As previously mentioned, a marked degree of attenuation was found in the culture maintained on glycerinated broth potato medium in the years from 1929 to 1933. This attenuation is shown graphically in curve A of Figs. 2 and 3.

The experiments described complete the cycle of variation in virulence for this bacterium. Calmette started with a virulent bovine strain and attenuated it in culture to obtain strain BCG. Watson (1928, 1933) obtained a virulent form from strain BCG, and the virulent derivative has since been attenuated until it is no more virulent than strain BCG. There is no direct evidence to explain these cycles, but the following suggestion seems worth making. The avirulent strain BCG is so unstable that it produces virulent Smooth types (Reed, Orr, and Rice 1934). These Smooth types became predominant when Watson injected the mixed cultures into guinea pigs and they consequently were reisolated as the prevalent type. However, such Smooth types are known to be unstable, and during the prolonged incubation in culture they produced attenuated strains that gradually replaced the virulent type.

JOHNE'S BACILLUS

Very little is known in regard to changes in virulence of *Mycobacterium paratuberculosis* or Johne's bacillus, the causative agent of paratuberculous enteritis of cattle. This lack of information is probably due to the facts that the disease is chronic and that the bacterium is not transmissible to small laboratory animals. The micro-organism was first isolated by Twort and Ingram in 1911 (1913). As a rule, it cannot be isolated and propagated unless the medium contains extracts from other acid-fast organisms such as *M. phlei* (Moeller) Bergey *et al.* which was found to be suitable for this purpose. However, some of

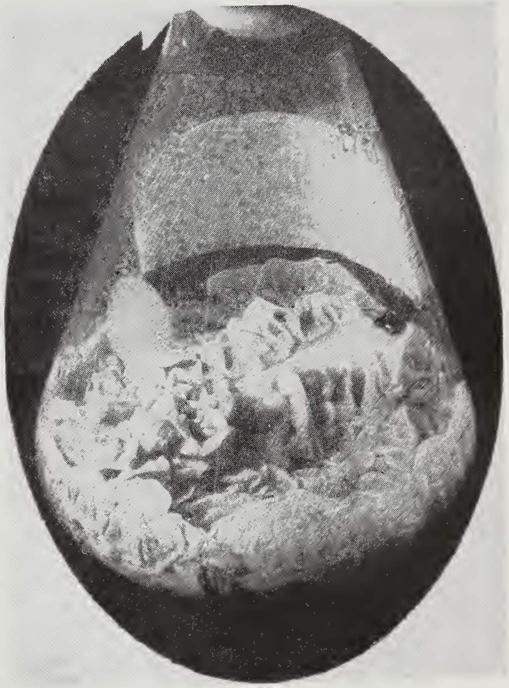

FIG. 7. The high folded smooth growth produced by the variant type of Johne's bacillus, strain No. 1, of Fig. 6, when cultured on potato cubes immersed in Long's synthetic medium.

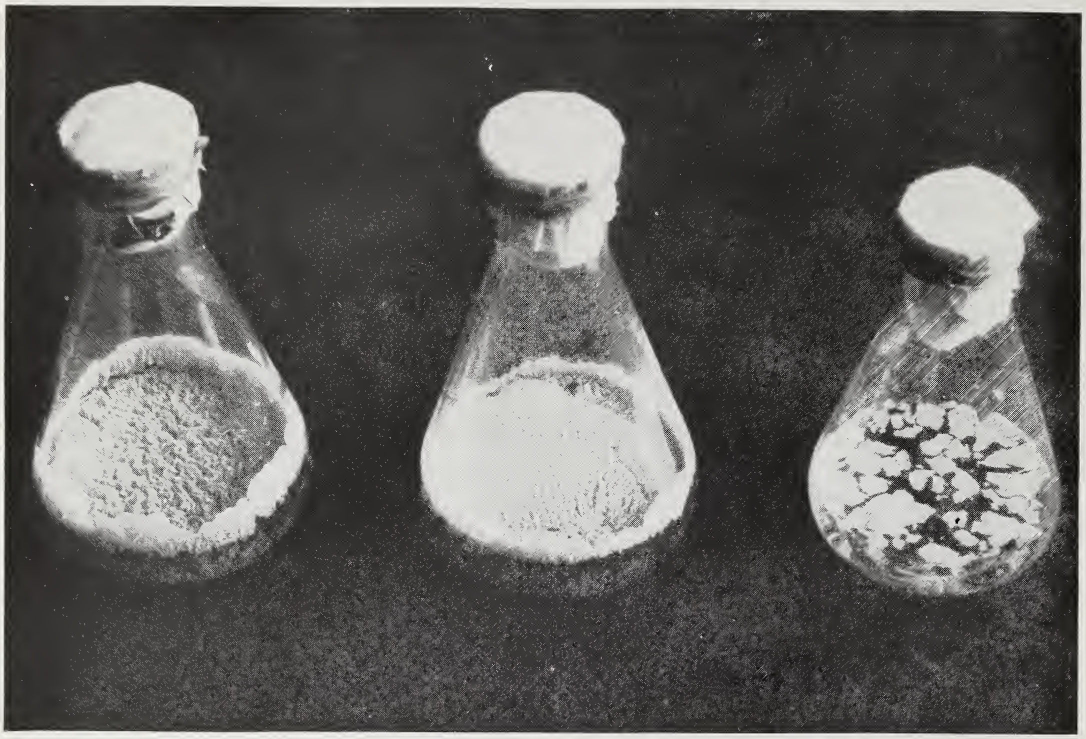

Fig. 8. Influence of H-ion concentration on certain strains of Johne's bacillus, other than strain No. 1. Three-month-old parent culture at pH 5.6 and 1½-month subcultures at pH 5.6 and 6.7, respectively, from left to right.

the older laboratory strains isolated by Twort and Ingram were observed to grow luxuriantly without these substances, but this fact has not been emphasized until recent years.

Some variability was observed in the cultural characteristics of our stock cultures of Johne's bacillus as it became adapted to growing on Long's synthetic medium, in the absence of *Mycobacterium phlei* extract. These changes are illustrated in Figs. 4 to 7. All cultures were grown on Long's plain synthetic medium or on potato surrounded by this medium. The character-

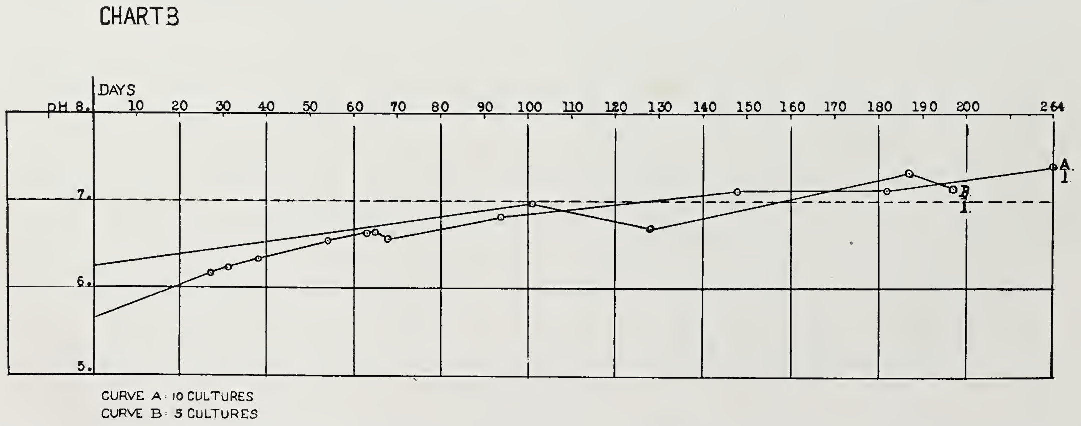

Fig. 9. Effect of cultures of the Smooth type of Johne's bacillus on pH, when grown in Long's synthetic medium. The Smooth type bacteria gradually render the medium more alkaline.

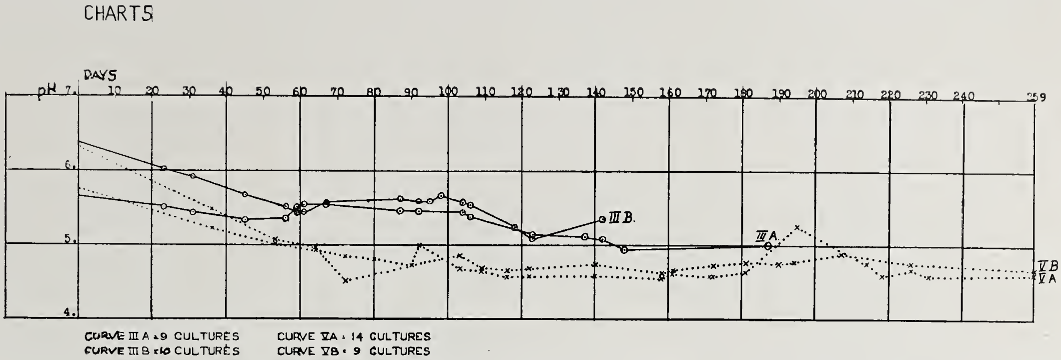

FIG. 10. Effect of cultures of the Rough type of Johne's bacillus on pH, when grown on Long's synthetic medium. The Rough type bacteria gradually render the medium more acid.

istic granular, knobby, folded growth seen in most of our strains, especially at pH 5.5 to 5.8, is shown in Fig. 4.

One of our strains (No. 1) gradually changed from the granular-knobby type into a smooth, thin, slightly glistening and folded island type of growth. This transformation was complete after approximately one year and occurred apparently under the modifying influence of an H-ion concentration of pH 6 to 6.4. Since 1932, all cultures of this strain have grown in a smooth, slightly folded fashion, particularly when on potato cubes immersed in Long's synthetic medium. The original granular growth is illustrated in Fig. 5, the smooth type on Long's synthetic medium in Fig. 6, and the folded smooth growth on potato in Fig. 7. An attempt to cause reversion to the granular growth by adjusting the medium to pH 5.5 to 5.8 and by adding *Mycobacterium phlei* extract was unsuccessful.

The influence of H-ion concentration on the character of growth in two other strains of our collection was noticeable and is illustrated very well by the cultures shown in Fig. 8. The center flask with medium at pH 5.6 has a granular folded growth, while the flask on the right with medium at pH 6.7 has smooth, thin, slightly glistening and mucoid islands. In the flask at the extreme left is shown the mature parent culture grown at pH 5.6.

There are many pleomorphic forms in the smooth growth of strain I on Long's synthetic medium. There is also a high percentage of cells that are not acid-fast or are only partially so. In granular cultures, on the other hand, the cell morphology is fairly uniform and there are few semi- or non-acid-fast individuals.

The smooth growth in Long's synthetic medium shown in Fig. 9 is remarkable in that it gradually renders the medium more alkaline. The granular, rough strains shown in Fig. 10 make the medium more acid. A similar trend of the reaction curves was found by Birkhaug (1935) in his experiments with the Smooth and Rough variants of the avian strain of the tubercle bacillus. In view of the close relationship between the avian tubercle bacillus and Johne's bacillus, this seems to be of some significance, suggesting that our smooth strain is a Smooth variant of Johne's bacillus and that granular strains are composed mainly of Rough variants which predominate under cultural conditions most suitable for a luxuriant growth.

Although comparative pathogenicity tests with Smooth and Rough cultures of Johne's bacillus were not possible, their alternative sensitizing power and the potency of their johnins was measured on guinea pigs. In a few preliminary experiments these properties of the smooth strain appeared to be of a lower degree than those of the granular strains.

SUMMARY

In the foregoing review an attempt has been made to illustrate the variability in virulence of bacteria for animals by discussing several important micro-organisms. The changes in pathogenicity have been correlated, wherever possible, with changes in physiological and morphological properties.

Many of these spontaneous variations have occurred in nature so that the modifying environmental factors are not well known. Such variations are, however, very important insofar as they affect both our study of epidemics and our concept of bacterial species.

Some of the variations known to occur in nature have been duplicated under rigidly controlled conditions in pure culture. By employing the variants as vaccines, it has been possible to develop various prophylactic measures that have greatly influenced the control of infectious diseases. Some of the best examples of the use of attenuated strains may be found in the brilliant work of Pasteur on anthrax, swine erysipelas, and fowl cholera.

References Cited

Arloing, S. 1885. Influence du soleil sur la végétabilité des spores du Bacillus anthracis. *Compt. rend. Acad. Sci.*, 101: 511.

Birkhaug, K. E. 1935. Etudes sur la dissociation du Mycobacterium tuberculosis. *Ann. Inst. Pasteur*, 54: 19, 195.

Breed, F. 1938. Swine Erysipelas. Its Distribution, Increasing Importance and Control. *J. Amer. Vet. M. A.* (n.s.), 45: 344.

Bujwid, O. 1919. Schutzimpfungen mit abgetöteten Bakterien bei Tierseuchen. *Zentralbl. f. Bakt., I, Orig.*, 82: 308.

Bulloch, W., Colebrook, L., Griffith, F. and St. John-Brooks, R. 1931. Actinomycosis and Similar Conditions. Chapt. II, *A System of Bacteriology*, Med. Res. Counc., London, 8: 71.

Buzna, D. 1925. Die Unterscheidung des vollvirulenten Milzbrandbazillus von den mitigierten (Vaccine-) Varietäten auf verschiedenen Kohlehydrat-Nährböden. *Zeitschr. f. Infektionskr. etc. d. Haustiere*, 28: 267.

Calmette, A. and Guérin, C. 1924. Vaccination des bovidés contre la tuberculose et méthode nouvelle de prophylaxie de la tuberculose bovine. *Ann. Inst. Pasteur*, 38: 371.

Calmette, A., Guérin, C. and Nègre, L. 1933. Etude expérimentale d'un bacille tuberculeux d'origine humaine cultivé pendant vingt-deux années consécutives sur milieu bilié. *Ann. Inst. Pasteur*, 50: 599.

Chamberland, Ch. and Roux, E. 1883. Sur l'atténuation de la virulence de la bactéridie charbonneuse, sous l'influence des substances antiseptiques. *Compt. rend. Acad. Sci.*, 96: 1088.

Claypole, E. J. 1913. On the Classification of the Streptothrices, Particularly in Their Relation to Bacteria. *J. Exp. Med.*, 17: 99.

Colebrook, L. 1921. A Report upon 25 Cases of Actinomycosis, with Especial Reference to Vaccine Therapy; a Discussion of Some Problems Connected with the Disease. *Lancet*, 1: 893.

Darzine, E. 1926. Action de la bile sur le bacille du rouget. *Compt. rend. Soc. Biol.*, 94: 625.

Edwards, J. T. 1931. Swine Erysipelas. Chapt. XII, *A System of Bacteriology*, Med. Res. Counc., London, 8: 379.

Eurich, F. W. and Hewlett, R. T. 1930. Bacillus Anthracis. Chapt. X, *A System of Bacteriology*, Med. Res. Counc., London, 5: 439.

Fosterman, A. A. 1932. Swine Erysipelas. *N. Amer. Vet.*, 13: 5.

Foulerton, A. G. R. 1910. The Milroy Lectures on the Streptotrichoses and Tuberculosis. *Lancet*, 1: 552, 626, 769.

———. 1912. As to the Nature of the Parasites of Leprosy and Tuberculosis. *Brit. Med. J.*, 1: 300.

Fulton, J. S. 1933. Swine Erysipelas. *Canad. J. Res.*, 8: 312.

Gomez, A. K. 1926. An Outbreak of Fowl Cholera. *J. Amer. Vet. M. A.* (n.s.), 21: 657.

Gotschlich, E. 1929. Allgemeine Morphologie und Biologie der pathogenen Mikroorganismen. *Handb. path. Mikroorg.*, 3rd ed., 1: 206. Fischer, Jena.

Griffith, A. S. 1924. Atypical Tubercle Bacilli in Human and Animal Tuberculosis with Special Reference to Those Occurring in Lupus. *Tubercle*, 5: 569.

Hadley, P. 1912. Studies on Fowl Cholera. I. A Biological Study of Ten Strains of the Fowl Cholera Organism. *Zentralbl. f. Bakt., I, Orig.*, 61: 323.

Hadley, P. 1913. Studies on Fowl Cholera. II. Active Immunity in Rabbits. *Zentralbl. f. Bakt., I, Orig.*, 69: 271.

Hadley, P. 1915. The Reciprocal Relations of Virulent and Attenuated Cultures in Active Immunization. *Zentralbl. f. Bakt., I, Orig.*, 76: 442.

———. 1927. Microbic Dissociation. The Stability of Bacterial Species with Special Reference to Active Dissociation and Transmissible Autolysis. *J. Infect. Dis.*, 40: 1.

Hegyeli, Z. 1931. A baktériumok virulentiájának fokozása állatpassage nélkül. (Increase in the Virulence of Bacteria without Animal Passage.) *Állatorv. Lapok* (Hungarian), 54: 185.

———. 1937. A Bac. anthracis virulentiájának fokozása és gyengítése táptalajokkal. (Increase and Decrease in the Virulence of Bac. Anthracis by Means of Culture Media.) *Állatorv. Lapok* (Hungarian), 60: 17.

Hutyra, F. v. and Marek, J. 1922. Infektionskrankheiten. Sp. Path. u. Therap. d. Haustiere, 6th ed., 1: 1, 76, 102, 576, 736. Fischer, Jena.

Kirchner, O. 1931. Zur Veränderlichkeit des Tuberkuloseerregers in morphologischer Hinsicht. *Beitr. z. Klinik. d. Tuberc.*, 77: 72.

Lange, L. 1932. Die Tuberkelbazillen. *Zentralbl. f. Bakt., I, Orig.*, 127: 10.

Mack, W. B. and Records, E. 1916. The Use of Bacterins in the Control of Fowl Cholera. *Agr. Exp. Sta. Univ. Nevada Bull.*, 85.

Manninger, R. 1919. Ueber eine Mutation des Geflügelcholerabazillus. *Zentralbl. f. Bakt., I, Orig.*, 83: 525.

Mitchell, C. A. and Duthie, R. C. 1929. Tuberculosis of the Common Crow. A preliminary study. *Amer. Rev. Tuberc.*, 19: 134.

Panisset, L. and Kolda, J. 1924. L'influence de la bile sur le bacille du rouget. *Compt. rend. Soc. Biol.*, 90: 343.

Petroff, S. A., Branch, A. and Steenken, W., Jr. 1929. A Study of Bacillus Calmette-Guérin (BCG). I. Biological Characteristics, Cultural ''Dissociation'' and Animal Experimentation. *Amer. Rev. Tuberc.*, 19: 9.

Preisz, H. 1904. Studien über Morphologie und Biologie des Milzbrandbacillus (mit besonderer Berüchsichtigung der Sporenbildung auch bei anderen Bacillen). *Zentralbl. f. Bakt., I, Orig.*, 35: 280.

————. 1911. Studien über das Variieren und das Wesen der Abschwächung des Milzbrandbacillus. *Zentralbl. f. Bakt., I, Orig.*, 58: 510.

Rabinowitsch, L. 1906. Ueber spontane Affentuberkulose, ein Beitrag zur Tuberkulosefrage. *Deutsch. med. Wochenschr.*, 32: 866.

Redlich, E. 1932. Glatte und rauhe Kolonien bildende Rotlaufbakterien im kranken Schwein. *Zeitschr. f. Infektionskr. etc. d. Haustiere*, 42: 300.

Reed, G. B., Orr, J. H. and Rice, C. E. 1934. Studies in Variability of Tubercle Bacilli; BCG. *Canad. J. Res.*, 11: 362.

Roy. Comm. Tuberc., Final Rept. 1911. H. M. Sta. Off., London.

Schütze, H. 1929. Pasteurella trevisan. Chapt. VI, *A System of Bacteriology*, Med. Res. Counc., London, 4: 446.

Sedlmeier, H. 1936. Kulturelle und experimentelle Untersuchungen über die Aetiologie der Aktinomykose des Rindes. *Zeitschr. f. Infektionskr. etc. d. Haustiere*, 50: 129.

Stang, V. 1901. Zur Kenntnis der Toxinbildung des Bact. avicidum. *Inaug. Dissert.*, Bern.

Topley, W. W. C. and Wilson, G. S. 1929. The Principles of Bacteriology and Immunity, 1: 242, 261; 2: 787, 799. Wm. Wood & Co., N. Y.

Twort, F. W. and Ingram, G. L. Y. 1913. A Monograph of Johne's Disease, Baillière, London.

Watson, E. A. (in collaboration with McIntosh, C. W. and Konst, H.). 1928. Researches on Bacillus-Calmette-Guérin and Experimental Vaccination against Bovine Tuberculosis. *J. Amer. Vet. M. A.* (n.s.), 26: 799.

Watson, E. A. 1933. Studies on Bacillus Calmette-Guérin (B.C.G.) and Vaccination against Tuberculosis. *Canad. J. Res.*, 9: 128.

Wright, J. H. 1904–05. The Biology of the Microorganism of Actinomycosis. *J. Med. Res.*, 13: 349.

BACTERIA PATHOGENIC ON PLANTS

By A. J. RIKER

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF WISCONSIN, MADISON, WIS.

Introduction

The bacteria causing diseases in plants are considered in a further discussion on the genetics of pathogenic organisms. In the evolutionary scale the bacteria comprise the lowest group. If some champion of a virus wishes to challenge this claim to distinction, we shall gladly yield in his favor and say that the bacteria are the lowest readily visible forms. One can see them with a good microscope, but that is about all. Even our sense of humor fails to contemplate both crossing *Erwinia amylovora* (Bur.) Winslow *et al.* with *E. carotovora* (Jones) Holl. and testing the progeny to see whether or not they would be "aggressors" against carrots in a three to one ratio. Present technique is hopelessly inadequate for such an attempt. But low as they are, the bacteria make up in activity for what they lack in size. Some of them show great variability, which makes the term "genetics" clearly applicable to them. Cole and Wright (1916) discussed the use of bacteria for genetic studies and emphasized long ago the importance of taking genetic concepts into account. Since the bacteria multiply at enormous rates—several times within an hour or over 2000 times in a month, under favorable conditions—they are surprisingly stable. As Brierley (1922) says, this ". . . implies an extremely delicate and constant hereditary mechanism, quite comparable in its behavioristic fixity and nicety to that in higher organisms." Although Mendelian ratios are beyond present reach, one can readily study the clear-cut variations which may occur frequently, and which are of great theoretical and practical importance.

Opportunity for Basic Work with Plant Pathogens. Investigations on variability in bacteria are fully justified merely from the standpoint of plants and plant diseases. In addition, many of the broad fundamental questions in biology regarding virulence, infection, immunity, and epidemiology can be approached through this group of bacteria with a facility worthy of special consideration. In the limited space available for this discussion, it seems better to emphasize points of view, opportunities, and requirements for future work along these basic biological lines than to give a detailed classification of variations among bacterial plant pathogens.

The common meeting grounds for the human, animal, and plant bacteriologists are very real, although not always immediately apparent. Some of the analogies drawn between animal and plant diseases—for example, in relation to "cancers" or "tumors"—have been severely criticized. Quite aside from the merits of these discussions, some of them have appeared to draw these groups of workers away from each other and to emphasize the differences between their subjects rather than the similarities. However, it seems so well accepted that many basic life processes in cells of plants and animals (including man) are similar that this point needs no discussion here. It is likewise unnecessary to elaborate on the axiomatic statement that a fundamental advance in one biological field very commonly assists in the development of related fields.

The mutual interests of the human, animal, and plant bacteriologists are very close, if not identical, in many fundamental subjects. Since these mutual interests are quite broad and have been treated extensively elsewhere (*e.g.*, Brierley 1922a), only some of the more important topics (see Brierley 1929) pertinent to our title are listed here. They include:

(1) Life cycles, referring to changes in the morphology of individual cells and the

relation of these different forms to virulence.

(2) Changes in colony characters and physiology, including particularly changes in pathogenicity.

(3) Factors attending changes, such as: time, frequency and conditions of origin, influence of environment, and relation to earlier and succeeding generations.

(4) Statistical analysis, to clarify the origin and frequency of the variations observed.

(5) Life histories of the pathogen in relation to entrance into the host, location, exit, and transmission to a new host, which are the essentials (Smith 1921) of studies in epidemiology, and which are so vitally influenced by variations in the pathogen.

The physical, chemical, and bacteriological techniques employed by the human, animal, and plant bacteriologists are essentially similar, differing only in minor modifications imposed by the host.

The experimental facility, provided by many plant diseases, deserves consideration for fundamental biological studies. The kinds of plant diseases available, and some of the experimental advantages they offer the technician, are worth mentioning. A list of over 150 bacterial plant pathogens, of which one or another attacks almost every economic plant, is given by Elliott (1930) with extensive literature citations. General discussions of the plant pathogens are presented by Link (1928), Smith (1920), and Stapp (1928, 1935). The symptoms in plants, caused by various bacteria, differ widely. Several classes of symptoms, which overlap more or less completely, are taken up briefly.

(1) *Bacterial galls.* Some bacteria stimulate growth. Those inducing galls on olives and sugar beets produce more or less conspicuous cavities between the host cells. The crown-gall organism, *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.,* produces inconspicuous cavities, or none at all, in the host and induces much hyperplasia and hypertrophy. This organism has a very wide host range. It is perhaps better understood than any of the other plant pathogens, because of the parallels between the associated pathological growths and those found in a well-known disease in higher animals. A discussion of crown gall in relation to basic biological research has been given by Riker (1939). Other bacteria related to the crown-gall organism produce galls, fasciations, or excessive root developments. The legume root-nodule bacteria, *Rhizobium* sp., enter through the root hairs and become established inside root cells, which then enlarge. The surrounding cells increase in number. Nodules, in which elemental nitrogen is fixed, are formed of varying sizes and shapes characteristic for the host plant and bacteria. Since this relation is beneficial, it removes these bacteria somewhat from the plant pathogens in classification. Because of this unique and economically important physiological property, this group has been studied a great deal. The literature is reviewed by Fred, Baldwin, and McCoy (1932). The bacteria producing other classes of symptoms, with perhaps occasional exceptions, have received much less critical study.

(2) *Bacterial wilt.* The vascular elements of certain plants are invaded by bacteria so that wilting results. In some cases, as with *Erwinia tracheiphila* (Smith) Holl. in cucurbits, the abundantly formed bacterial gum may cause a mechanical obstruction. In others, the toxic action of the bacteria makes the picture less clear-cut. Recent studies on variation in the causal organism of corn wilt, *Phytomonas stewartii* (Smith) Bergey *et al.,* are discussed later. A vascular invader in cabbage, *P. campestris* (Pam.) Bergey *et al.,* is important, particularly because it opens the way for soft-rot organisms.

(3) *Bacterial soft rot.* A group of bacteria, which produce pectinase and thus dissolve the material holding the cells together, reduce fleshy plant tissue to useless and often odorous slime. *Erwinia carotovora* is an example of this type. Variability in this group is reported by Leach (1930) and Stanley (1938). Rather similar activity in stems and leaves, perhaps be-

cause of structure, gives different symptoms.

(4) *Cankers and leaf spots.* The largest group of bacterial plant pathogens includes those which cause spots on leaves and cankers on stems. *Phytomonas phaseoli* (Smith) Bergey *et al.*, and *Erwinia amylovora,* which cause bean and pear blights, respectively, are examples. Some organisms in this group, such as *P. tabaci* (W. and F.) Bergey *et al.*, produce a toxic substance which diffuses into the tissue some distance from the bacteria. This substance, which is heat stable, is also produced on culture media. Thus the different symptoms caused by plant pathogens give a wide choice of problems for basic investigations.

Some of the more technical advantages of working with plants are outlined. For example:

(1) Large numbers are easily available. The number used, whether ten, a hundred, or a thousand, is commonly adjusted to experimental needs.

(2) The initial cost and the expense of maintaining plants are very low in comparison with those of animals.

(3) The species of plants attacked frequently contain varieties or selections possessing several degrees of resistance and susceptibility.

(4) Plants are suited to a wide range of experimental procedures, many of which are not feasible with animals.

(5) Epidemics are induced with relative ease and can be studied without concern for the health of the technicians or the public.

(6) The genetic purity of the host can be assured. Seed from long lines of successively self-fertilized parents are already available in many varieties of plants. However, when this is not sufficient, one can commonly find, or easily develop, experimental units all genetically identical through vegetative propagation. For example, within a named variety of many fruits and ornamentals, the numerous individuals are all vegetative parts of one original selected parent. Except for occasional bud variations, they are all genetically the same. This is particularly advantageous since pathogenicity is necessarily defined in terms of susceptibility of the host.

For the sake of an unsanguine technician, it might be added that plants do not squeal, bite, or otherwise create an atmosphere suggesting an unsympathetic and disorganized world.

These rather general comments about the opportunities for research can best be followed by a few selected examples chosen with no pretense at covering the bacterial-disease literature. Among them are included some about the legume root-nodule bacteria without, of course, an implication that they produce disease. The extensive research on their well-known symbiotic relationships adds breadth and strength to the general topic.

Variations and Circumstances of their Appearance. Variations in common bacteriological characters of the plant pathogens have frequently been observed. The changes in cell shape, where studied, are similar to those reported by Henrici (1928). Such changes in the legume root-nodule bacteria are well known (Fred *et al.*, 1932). Differences in colony characters have been concerned primarily with color, consistency, phase (especially Rough and Smooth), and the formation of sectors. Alterations in such characters as sugar fermentation, nitrate reduction, and serum reactions are also relatively common. Cottrell-Dormer (1936) concluded that every species contains a number of more or less well differentiated strains, and that new genotypic forms arise from time to time. In some cases variations have been correlated with pathogenicity, while in others they have not. However, it seems probable that, when a culture shows variability in cultural characters, changes in virulence may also be expected. Pathogenicity is a notoriously unstable character for certain plant bacteria (Stapp 1935), but at the same time it is an outstanding character which makes these organisms interesting and important.

The value to be placed on the reports of variations among the bacteria pathogenic on plants involves many considerations. As we look over the literature, for example, as assembled by Elliott (1930), we are impressed by the many conflicting reports on bacteriological characters. Perhaps one should approach this problem humbly and with Huxley's prayer on his lips, "God, give me courage to face a fact though it slay me." These are some of the disconcerting facts one faces. The variations reported for these bacteria have appeared, for example, because: (1) the bacteria themselves actually have varied, (2) the cultures used have been mixtures of several strains or even species of bacteria instead of pure cultures, (3) different methods have been employed for determining the same character, (4) the same methods have been employed under different conditions, which influenced results, and (5) the same results have been interpreted differently by the several investigators. If we consider the crown-gall organism as an example, it is possible to find one "authority" for saying it is positive and another for saying it is negative with respect to most of the common bacteriological characters. In the earlier literature there is surprising agreement that this organism produces no gas from glucose or sucrose. Yet Conner *et al.* (1937), with adequate methods, found that it produced from .02 to .05 gm of carbon dioxide per 100 cc of medium, depending on conditions. We considered this species extremely variable until we adopted single-cell technique as a laboratory routine and eliminated contaminations or mixtures from our cultures. The use of mixed cultures probably has caused more apparent variation than any other single factor.

The purity of the bacterial culture is of paramount importance in studies on variability in pathogenicity. In the bacteriological brood of ducks the question continually arises concerning the status of an ugly duckling. Will it become a swan-like substantiation of a beautiful working hypothesis about variation, or will it turn out a mud hen demonstrating only an error in technique? In many routine procedures, any cultures that look "different" can be discarded as contaminations. However, when variations are being sought, some means must be provided for differentiating between *bona fide* variants and "condamnations," as the boys call them. It is usually recognized that those who rely on the common poured-plate technique have a false sense of security. The interpretations based on single-colony technique are sometimes quite different from those based on the same cultures when single-cell technique is added. For example, the evidence (Riker *et al.* 1930) from single-colony cultures of crown-gall and hairy-root bacteria, even after four successive replatings, suggests that they intergrade through apparently intermediate cultures. However, supplementary single-cell cultures showed that the "intermediates" studied were mixtures (Riker *et al.* 1930; and Wright *et al.* 1930). Similarly, among the legume root-nodule bacteria, the pea and clover organisms were separated with certainty only by single-cell isolations (Baldwin, correspondence). The use of single-cell cultures (*e.g.*, Brierley 1929; and Frobisher 1933) would provide an easy answer to questions regarding the original unity of bacteria, if only they were not so small. Many attempts to provide a satisfactory technique have resulted in an extensive literature reviewed by Dickinson (1933), Reyniers (1933), and Hildebrand (1938). While the procedures are not yet fool proof, they appear to be indispensable adjuncts to the poured-plate technique. Although single-colony and single-cell cultures are frequently indistinguishable, differences occur so often that single-cell isolation is a routine in our laboratories, and is always employed where questions of variations appear.

While purity of cultures is essential for many studies, it may be recalled that mixtures of organisms are the rule in nature and deserve investigation. Variations in the disease-producing activity of a bacterium may occur, not because of an inherent change in the bacterium itself, but because of differences in its association with some other organism. For example, the blackleg organism causes little damage on potato if there is no corn maggot to break down barriers of cork tissue. The damage from *Phytophthora* late blight on potato would be much less severe if there were no secondary soft-rot organisms. The role of black rot of cabbage in providing an infection court for soft-rot bacteria has already been mentioned. Examples of bacterial association with an insect, a fungus, or another bacterium would not be complete without mentioning the bacteriophage—if it can be done without commitment that the phage is living. In the literature, brought together by Katznelson (1937), one finds claims that a phage may cause, increase, or decrease the pathogenicity of plant bacteria. The whole question of change in pathogenicity in relation to antagonistic and synergetic relations between pathogenic agents acting together appears to carry great research opportunity. The influence of such interactions on variations among the bacterial pathogens is practically untouched.

The difficulties accompanying any attempt to evaluate the variations reported in the literature, the time available, and the enemies we might create, make it unwise to present a critical catalogue of the observed changes. Instead, and with no attempt to review the literature, let us examine some examples illustrating particularly alteration in pathogenicity and a few of the attending circumstances.

A slow change in pathogenicity has been reported for *Phytomonas stewartii.* Wellhausen (1937) increased the virulence in single-colony cultures of this species by successive passages ("... 10–15 colonies pooled constituted the inoculum for the next passage") through resistant sweet corn plants and lowered the virulence by similar passage through susceptible plants. If substantiated by further work, this is highly significant because it is contrary to the usual findings with animal pathogens. Virulence is commonly reduced in resistant animals and increased in susceptible animals (Arkwright 1930). Similar changes in virulence of *P. stewartii* have been reported by McNew (1938) with single-colony cultures purified by repeated plating and grown in media with and without organic nitrogen. He says, "These changes in virulence . . . apparently were brought about as the result of an intensive selection of variants that were produced by the slightly virulent culture." With a number of single-cell cultures of Stewart's wilt bacterium, Ivanoff *et al.* (1938) reported differences in physiology and pathogenicity. Some types were relatively stable while others seemed to change slowly. Lincoln (1939) has shown that, in line with Wellhausen's report, the virulent bacteria are favored in the resistant plants and the attenuated bacteria are favored in the susceptible plants. For his work, Lincoln mixed a single-cell virulent culture with a single-cell attenuated culture, inoculated plants, and studied the relative proportion of each culture before and after plant passage.

These investigations by Lincoln indicate that two problems need clearly to be differentiated in work on the "genetics" of bacterial pathogens. In the first problem, there are mixed together two or more strains of bacteria which are different in relation to the character being studied. In one host or culture medium one strain outgrows the others, while in another host or medium, some different strain predominates. A character such as pathogenicity might increase or diminish, as Lincoln shows, depending on the predominance of one strain or another. Such information is valuable, but really is quite different from the type of variation we are discussing. The purity of the original cultures is obviously essential for eliminating confusion between this phenomenon and variation in an originally pure culture. In this second problem, there is only one strain of a bacterial pathogen at the beginning. The variations which occur in its progeny are of fundamental biological importance and deserving of exhaustive study. The two problems are intimately related because, when one starts with a "pure" culture and then finds that a *bona fide* variation has occurred, one desires to learn how the two cultures interact. However, it is with the second of these two problems that we are primarily concerned.

Slow changes in pathogenicity have also been found for cell-stimulating cultures. With legume root-nodule bacteria, passage of homologous strains through red clover, soybeans, peas, and alfalfa, respectively, may increase or diminish effectiveness depending on the strain (Allen and Baldwin 1931), as shown in Fig. 1. Legume cultures are reduced in effectiveness by growth on glycine media (Longley *et al.* 1937). This is also the case with crown-gall cultures. Single-cell crown-gall cultures, which have been constant in virulence for 10 years, have been attenuated (see Fig. 2) by 10 to 20 successive transfers in glycine and related compounds (Riker *et al.* 1938). Frequent use of single-cell technique was made throughout the work, in order to avoid, as far as possible, any mixture of cultures. In more recent unpublished work, attenuated cultures have been restored practically to full virulence by cultivation on routine yeast-infusion-sugar media. More than half a dozen compounds related to glycine have similar attenuating effects, while other related substances do not. So far, there seems to be no connection between the structure of the amino compounds and capacity for attenuation. It will be interesting to study whether these materials which prevent the pathological growth of crown-gall cells will also influence the pathological growth of certain animal cells.

Slow loss of pathogenicity in cultures from other species is commonly reported in the literature. For example, Braun (1937) found that single-cell cultures of the tobacco wild-fire organism, *Phytomonas tabaci,* slowly lost the capacity for producing a toxic substance. Frequent transfers on beef-extract-peptone-sugar media brought about the change more rapidly than occasional transfers. A correlation is suggested between rate of growth and change. However, this variation apparently did not influence the ability of the bacteria to invade tobacco leaf tissue. Ark (1937) ob-

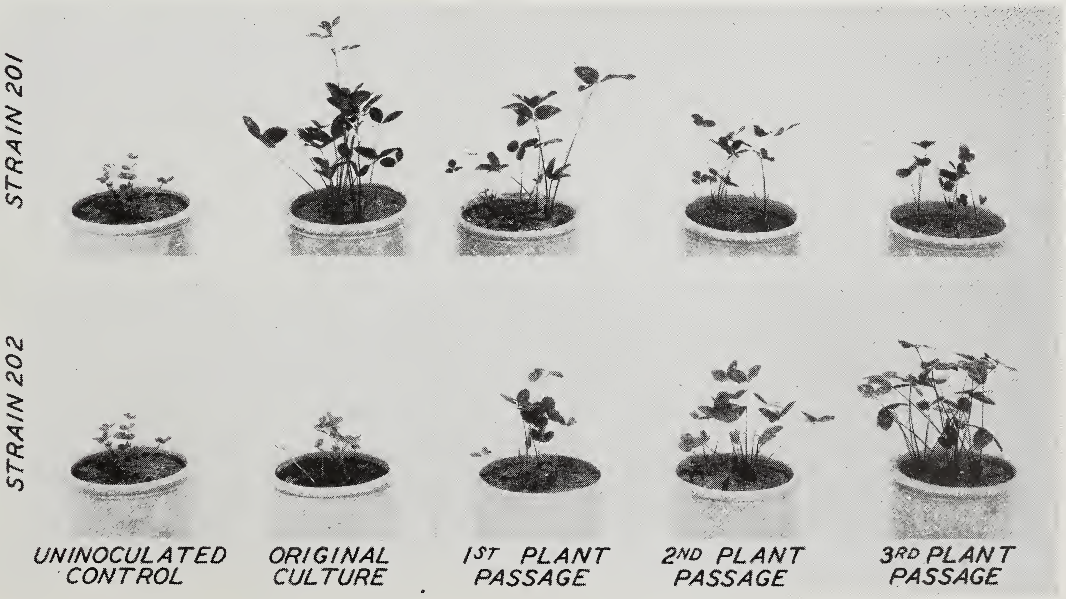

Fig. 1. Effects of successive plant passage through red clover on the legume root-nodule bacteria. The cultures were passed through plants, recovered, placed in stock, and transfers passed again successively through other plants. Original stock cultures and later subcultures which had passed 1, 2, and 3 times through plants were all employed in a parallel series with uninoculated controls. Within limits, strain 201 decreased in effectiveness and strain 202 increased. (After Allen and Baldwin 1931.)

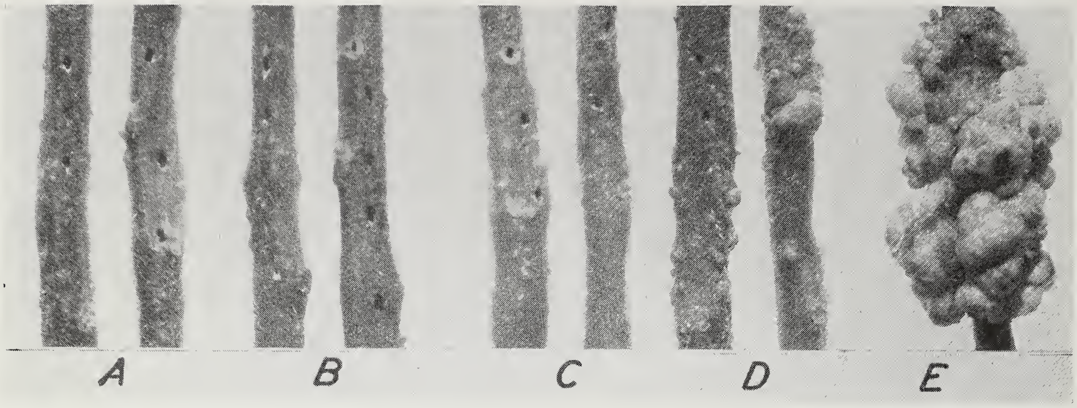

FIG. 2. Results of inoculations on tomato stems with cultures grown 15 to 20 successive transfers in media containing, respectively, (A) 0.3 per cent glycine, (B) 1.0 per cent glycylglycine, (C) 2.4 per cent alanine, (D) 2.7 per cent dicyandiamide, and (E) 5.0 per cent potassium nitrate. A, B, and C show attenuation, D, partial attenuation, and E, no attenuation.

served an increase in virulence in single-cell fire-blight cultures, *Erwinia amylovora,* during successive passages through 2 per cent sucrose broth, and a decrease in virulence during passages through 10 per cent sucrose broth. While slow changes in single-cell cultures occur, individual cells in these cultures may undergo rapid changes which are slow in making their appearance in the activity of the whole culture.

A rapid change in character, sometimes accompanied by a change in virulence, has been shown by several lines of work. Sectors, which indicate a sudden change, have been observed in different species of bacteria. For example, Nirula (1928) found saltations in several single-cell cultures. In spite of repeated trials, he found no reversions. Bryan reported sectors lower in virulence in single-colony cultures pathogenic on tomato (1931) and cucumber (1932). Cottrell-Dormer (1936) found sectors in single-colony cultures of 3 species attacking sugar cane. Hendrickson *et al.* (1934) showed that single-cell crown-gall culture, when grown on an aniline blue medium, had frequent sectors. It was possible by selection to develop cultures which did not absorb the dye. Other rapid changes in cultural characters occurred in relation to plant passage. An attenuated variant, which was found by using dilution plates, was purified by single-cell technique. It appeared identical to the mother culture in its bacteriological characters (Lyneis *et al.* 1936), but differed in pathogenicity (Locke *et al.* 1938). Duggar and associates (unpublished), in preliminary trials with single-cell crown-gall cultures, partially attenuated by glycine, found that irradiation influenced pathogenicity. When most of the bacteria were killed (all but about 0.1 per cent), the survivors on the average were somewhat more virulent than the non-irradiated bacteria. Thus, in some cases at least, changes in virulence, as well as in other characters, appear to be relatively sudden.

The change of colony characters from Smooth to Rough is also frequently rapid and is apparently associated with the character of the bacterial gum. The Smooth colonies often appear to be more virulent than the Rough colonies. In some cases at least this may be associated with rate of growth. Ark (1937), employing single-cell cultures, found that the Smooth form of *Erwinia amylovora* was distinctly more virulent than the Rough form. This Rough form appeared in old cultures and old plant lesions but could be changed to the Smooth form by 4 to 6 successive rapid transfers in a favorable medium. Ivanoff *et al.* (1938), with single-cell cultures of *Phytomonas stewartii,* reported that the more slimy cultures were commonly more virulent than the cultures with little slime. Berge *et al.* (1936) found that virulent single-cell crown-gall cultures were more viscous either in liquid or agar culture than the single-cell attenuated cultures. Conner *et al.* (1937) showed that 70 to 80 per cent of the sugar fermented by crown-gall bacteria was utilized in the formation of unidentified products related to gum. These and other similar studies indicate that the gum-like bacterial metabolites are worthy of further study in relation to pathogenicity and variation.

The plant pathogens are relatively constant under certain favorable conditions and relatively variable under others, particularly those preventing rapid vegetative growth. Aside from a few instances, such as those mentioned earlier, the changes are not predictable. A variety of cultural conditions, as discussed by Almon and Baldwin (1933) for legume root-nodule bacteria and by Ark (1937) for fire-blight bacteria, may be important for inducing variations among the plant pathogens. In a very real sense the living host tissue may be regarded as one type of medium for bacterial growth. So, since virulence is defined in terms of resistance or susceptibility of the host, the host plant deserves consideration in connection with changes in pathogenicity.

Appearance of Variations Caused by the Host. A physiological change in the host plant may give the appearance of variation in the bacteria. Riker (1926) found that tomato plants inoculated with crown-gall bacteria became infected at 28° C air temperature but not at 31° C. Both culture and host grew well at the higher temperature. Tomato plants bearing fruit produced smaller galls after inoculation than parallel plants from which the fruits had been removed (Stapp and Bortels 1931; and others). The position of inoculations on tomato with attenuated crown-gall cultures greatly influenced the result (Locke *et al.* 1938). Inoculations on a stem, which received no other treatment, gave little or no response. When the top of the plant was cut off and both the leaves and the axillary buds were removed from the upper remaining nodes, inoculations on the top of the stem produced a gall with adventitious leaves and shoots. If a virulent crown-gall culture was placed on the top of such a decapitated plant, and inoculations made below with an attenuated culture, a growth substance apparently passed down the stem and stimulated the tissue about the at-

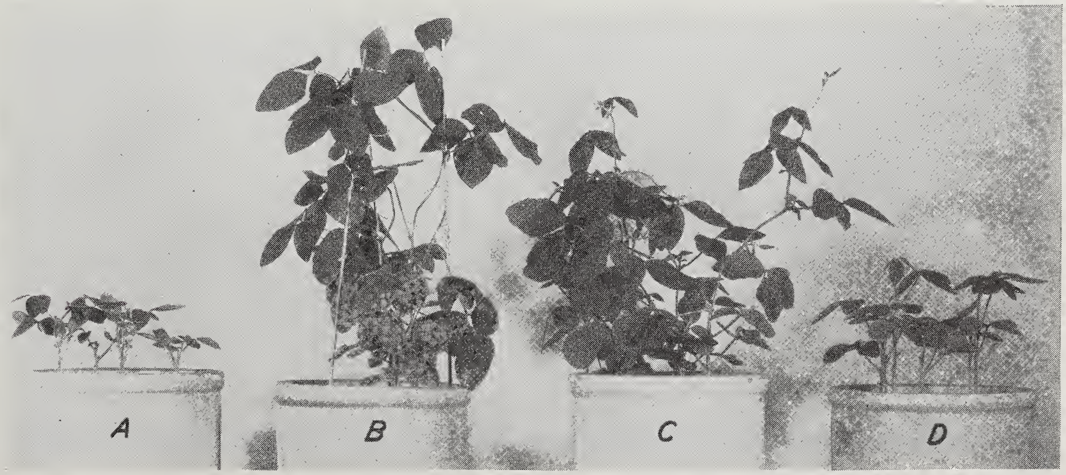

FIG. 3. Effect of shading on nodulated soybeans in which excessive carbohydrate in the carbohydrate-nitrogen balance inhibited the nitrogen fixation. (A) no shade; (B) in shade continuously for 33 days before harvest; (C) in shade for 12 days, returned to sun for 21 days; (D) in shade continuously for 15 days before harvest. (After Fred, *et al.* 1938.)

tenuated culture. McNew and Spencer (1939) found that nitrogen-deficient sweet-corn seedlings were not severely wilted by Stewart's wilt bacteria. Inoculated legume plants, when grown under several different conditions, respectively, so as to modify the usual carbohydrate-nitrogen ratio, have shown a great variation in response to homologous cultures. The variations occurred because of differences in plants rather than differences in cultures. Wilson (1937) says, "It was possible to develop a hypothesis ascribing a regulatory mechanism in symbiotic nitrogen fixation to the balance between carbohydrate and nitrogen in the host plant." Better nitrogen fixation occurred (Fred *et al.* 1938) in midsummer with reduced light (Fig. 3) or with added nitrogen than under ordinary conditions, within the limits necessary for a reasonable nutritional balance.

In connection with bacterial variation, Wilson *et al.* (1937a) say, "It is proposed that the concept of strain variation in the [legume root-nodule] bacteria may be only one aspect of a broader type of variation which involves the total relationship between organism and plant. . . . According to this view a given strain of the organism is not 'good' or 'poor' in an absolute sense, but only relative to the other factors which affect the relationship." It seems probable that this general concept can be broadened to include all the plant pathogens. Thus, a study on variations in pathogenicity must take into account such factors as the natural resistance of the host, its physiological condition, and the local environment.

In conclusion, one word of caution seems appropriate for any beginner who might choose to study some of the fascinating problems dealing with variability. Probably no field of biological study contains so many chances for error, both in technique and in the interpretation of results. With all the recent technological advances, the best possible procedure and the most exacting conclusions are not too good. This is no field for a "lone wolf," because only the most up-to-date methods should be employed, and not only the results but also their meaning need frequently to be checked by qualified colleagues. Only thus can we hope to build firmly and then eventually, at long last, to gain the kindly tolerance from future investigators which is often held now for those already gone.

Summary

Let us review briefly the essential points in this discussion. It appears that the investigators dealing with variability in bacteria causing diseases of men, animals, and plants have many common interests. Certain types of fundamental research can progress better with plants than with animals, because the plants offer many experimental advantages. The bacteria pathogenic on plants produce a wide range of symptoms on many different kinds of hosts and show a considerable range of variability. The original purity of a bacterial culture is essential for studies on variations. In some cases the changes in virulence discussed appear gradually, indicating that cultures can be "educated." In others, these changes appear to be relatively sudden and complete. Both kinds of change suggest "genetic" problems of scientific interest and basic importance which, when solved, will doubtless have valuable biological applications. Since pathogenicity must be defined in terms of the susceptibility of the host, a concept of variation in the disease-producing ability of bacteria must consider not only the genetic constitution but also the physiological condition and the environment of the host.

References Cited*

Allen, O. N. and Baldwin, I. L. 1931. The Effectiveness of Rhizobia as Influenced by Passage Through the Host Plant. *Wisc. Agr. Exp. Sta. Res. Bull.*, 106.

Almon, L. and Baldwin, I. L. 1933. The Stability of Cultures of Rhizobium. *J. Bact.*, 26: 229.

Ark, P. A. 1937. Variability in the Fire-blight Organism, Erwinia amylovora. *Phytopath.*, 27: 1.

Arkwright, J. A. 1930. Variation. In: *A System of Bacteriology*, Med. Res. Counc., London, 1: 311.

Berge, T. O., Riker, A. J. and Baldwin, I. L.

1936. Correlation of Pathogenicity and "Viscosity" in Cultures of Phytomonas tumefaciens. (Abst.) *Phytopath.*, 26: 86.

Braun, A. C. 1937. A Comparative Study of Bacterium tabacum Wolf and Foster and Bacterium angulatum Fromme and Murray. *Phytopath.*, 27: 283.

Brierley, W. B. *et al.* 1922. Discussion on Mutation of Species. *Brit. Med. J.*, 1922(2): 722.

Brierley, W. B. 1922a. [Discussion of paper by Lang, W. H., on] Some Aspects of Vegetable Pathology in Relation to Human Disease. *Brit. Med. J.*, 1922(2): 962.

————. 1929. Variation in Fungi and Bacteria. *Proc. Intern. Congr. Plant Sci., Ithaca*, 2: 1629.

Bryan, Mary K. 1931. Color Variations in Aplanobacter michiganense. *Phytopath.*, 21: 559.

————. 1932. Color Variations in Bacterial Plant Pathogens. *Phytopath.*, 22: 787.

Cole, L. J. and Wright, W. H. 1916. Application of the Pure-line Concept to Bacteria. *J. Infect. Dis.*, 19: 209.

Conner, H. A., Riker, A. J. and Peterson, W. H. 1937. The Carbon Metabolism of the Crown-gall and Hairy-root Organisms. *J. Bact.*, 34: 221.

Cottrell-Dormer, W. 1936. The Variability of Plant Pathogens. *Proc. Intern. Soc. Sugar Cane Techn.* (5th Congr., Brisbane), 1935: 712.

Dickinson, S. 1933. The Technique of Isolation in Microbiology. *Phytopath.*, 23: 357.

Elliott, Charlotte. 1930. Manual of Bacterial Plant Pathogens. Williams and Wilkins, Baltimore.

Fred, E. B., Baldwin, I. L. and McCoy, E. 1932. Root Nodule Bacteria and Leguminous Plants. *Wisc. Univ. Studies Sci.*, No. 5.

Fred, E. B., Wilson, P. W. and Wyss, O. 1938. Light Intensity and the Nitrogen Hunger Period in the Manchu Soybean. *Proc. Acad. Natl. Sci. Philadelphia*, 24: 46.

Frobisher, M., Jr. 1933. II. Some Pitfalls in Bacteriology. *J. Bact.*, 25: 565.

Hendrickson, A. A., Baldwin, I. L. and Riker, A. J. 1934. Studies on Certain Physiological Characters of Phytomonas tumefaciens, Phytomonas rhizogenes and Bacillus radiobacter. Part II. *J. Bact.*, 28: 597.

Henrici, A. T. 1928. Morphologic Variation and the Rate of Growth of Bacteria. Charles C. Thomas, Springfield, Illinois.

Hildebrand, E. M. 1938. Techniques for the Isolation of Single Micro-organisms. *Bot. Rev.*, 4: 627.

Ivanoff, S. S., Riker, A. J. and Dettwiler, H. A. 1938. Studies on Cultural Characteristics, Physiology and Pathogenicity of Strain Types of Phytomonas stewarti. *J. Bact.*, 35: 235.

Katznelson, H. 1937. Bacteriophage in Relation to Plant Diseases. *Bot. Rev.*, 3: 499.

Leach, J. G. 1930. The Identity of the Potato Blackleg Pathogene. *Phytopath.*, 20: 743.

Lincoln, R. E. 1939. Host-parasite Interactions with Bacterial Wilt of Maize. *Science* (n.s.), 89: 159.

Link, G. K. K. 1928. Bacteria in Relation to Plant Diseases. *In:* Jordan, E. O., and Falk, I. S., Editors. The Newer Knowledge of Bacteriology and Immunology. Univ. of Chicago Press, Chicago.

Locke, S. B., Riker, A. J. and Duggar, B. M. 1938. Growth Substance and the Development of Crown Gall. *J. Agr. Res.*, 57: 21.

Longley, B. J., Berge, T. O., Van Lanen, J. M. and Baldwin, I. L. 1937. Changes in the Infective Ability of Rhizobia and Phytomonas tumefaciens Induced by Culturing on Media Containing Glycine. (Abst.) *J. Bact.*, 33: 29.

Lyneis, Mary M., Riker, A. J. and Locke, S. B. 1936. Comparative Physiology of Pathogenic and Non-pathogenic Crown-gall Bacteria. (Abst.) *Phytopath.*, 26: 100.

McNew, G. L. 1938. The Relation of Nitrogen Nutrition to Virulence in Phytomonas stewarti. *Phytopath.*, 28: 769.

McNew, G. L. and Spencer, E. L. 1939. Invasiveness of Phytomonas stewarti in Sweet Corn Supplied with Different Amounts of Nitrogen. (Abst.) *Phytopath.*, 29: 17.

Nirula, R. L. 1928. Saltations in Bacteria. *Ann. Bot.*, 42: 431.

Reyniers, J. A. 1933. Studies in Micrurgical Technique. I. The Adaptation of Single Cell Technique to Routine Use. *J. Bact.*, 26: 251.

Riker, A. J. 1926. Studies on the Influence of Some Environmental Factors on the Development of Crown Gall. *J. Agr. Res.*, 32: 83.

————. 1939. Physiological Relations between Host and Parasite in Crown Gall—An Example of Basic Biological Research with Plant Materials. *Amer. J. Bot.*, 26: 159.

Riker, A. J., Banfield, W. M., Wright, W. H., Keitt, G. W. and Sagen, H. E. 1930. Studies on Infectious Hairy Root of Nursery Apple Trees. *J. Agr. Res.*, 41: 507.

Riker, A. J., Van Lanen, J. and Baldwin, I. L. 1938. Attenuation of Crown-gall Bacteria. (Abst.) *Phytopath.*, 28: 19.

Smith, E. F. 1920. An Introduction to Bacterial Diseases of Plants. W. B. Saunders Co., Philadelphia and London.

Smith, T. 1921. Parasitism as a Factor in Disease. *Science*, 54: 99.

Stanley, A. R. 1938. Physiologic and Serologic Studies of the Soft-rot and Colon Group of Bacteria. *West Virginia Agr. Exp. Sta. Bull.*, 287.

Stapp, C. 1928. Schizomycetes (Spaltpilze oder Bakterien). *In:* Sorauer, P. Handbuch der Pflanzenkrankheiten. Ed. 5, 2(1): 1. Paul Parey, Berlin.

————. 1935. Contemporary Understanding of Bacterial Plant Diseases and Their Causal Organisms. *Bot. Rev.*, 1: 405.

Stapp, C. and Bortels, H. 1931. Der Pflanzenkrebs und sein Erreger, Pseudomonas tumefaciens. I. Mitteilung: Konstitution und Tumorbildung der Wirtspflanze. *Zeitschr. Parasitenk.,* 3: 654.

Wellhausen, E. J. 1937. Effect of the Genetic Constitution of the Host on the Virulence of Phytomonas stewarti. *Phytopath.,* 27: 1070.

Wilson, P. W. 1937. Symbiotic Nitrogen-fixation by the Leguminosae. *Bot. Rev.,* 3: 365.

Wilson, P. W., Burton, J. C. and Bond, V. S. 1937a. Effect of Species of Host Plant on Nitrogen Fixation in Melilotus. *J. Agr. Res.,* 55: 619.

Wright, W. H., Hendrickson, A. A. and Riker, A. J. 1930. Studies on the Progeny of Single-cell Isolations from the Hairy-root and Crown-gall Organisms. *J. Agr. Res.,* 41: 541.

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* Since the Ottawa meeting considerable work bearing on this subject has been performed. As examples the following papers are cited:

Baldwin, I. L. 1939. Chemical Attenuation of Crown Gall Bacteria. *3rd Intern. Cong. Microbiol.* Abstracts of Communications. p. 234. (Abstract.)

Duggar, B. M. and Riker, A. J. 1940. The Influence of Ultraviolet Irradiation on the Pathogenicity of *Phytomonas tumefaciens. Phytopath.,* 30: 6. (Abstract.)

Hedges, Florence. 1940. Association of *Bacterium phaseoli* and the Virus of Common Bean Mosaic. *Phytopath.,* 30: 9. (Abstract.)

Hildebrand, E. M. 1940. Strains of the Fire Blight Organism. *Phytopath.,* 30: 9. (Abstract.)

Lincoln, Ralph E. 1940. Production and Rate of Mutation in *Phytomas stewartii* by X-radiation. *Genetics,* 25: 125–126. (Abstract.)

McNew, George L. and Spencer, Ernest L. 1939. Effect of Nitrogen Supply of Sweet Corn on the Wilt Bacterium. *Phytopath.,* 29: 1051.

Riker, A. J. and Baldwin, I. L. 1939. The Efficiency of the Poured Plate Technique as Applied to Studying Bacterial Plant Pathogens. *Phytopath.,* 29: 852.

Thomas, Roy C. 1940. Additional Facts Regarding Bacteriophage. *Phytopath.,* 30: 24. (Abstract.)

THE GENETICS OF THE PARASITIC PROTOZOA

By WILLIAM H. TALIAFERRO and CLAY G. HUFF
DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY, THE UNIVERSITY OF CHICAGO, CHICAGO, ILL.

A REVIEW of the genetics of parasitic protozoa is of value chiefly to emphasize the small amount of analytical work that has been done and the great importance of the field for further investigation. Results so far obtained agree closely with those secured with free-living protozoa and suggest that parasites may offer unique material for the solution of certain genetic problems provided such technical obstacles as difficulties in control of the parasite after it has entered the host, in observation of individual organisms, and in acquiring knowledge of the cytology of the heredity-bearing portions of the cell can be overcome.

Undoubtedly, various strains of parasitic protozoa within a given species vary among themselves in many structural and physiological characteristics; some of these characteristics seem to be induced environmentally and may be inherited for long periods. Experimental data are, however, entirely inadequate to differentiate phenotypic from genotypic variations, so that the use of such terms as mutation, phenotype, and genotype are unwarranted if they are meant to have the same connotation as with the higher plants and animals. We have followed Jennings (1929) in designating a group of individuals, of the same hereditary constitution, a biotype, without attempting to decide the exact genetic basis of this inherited constitution. As Jennings points out, there may be transient and permanent biotypes. Where the biotype has seemed to be environmentally induced, we have used Jollos' (1921) term, *Dauermodifikation* or persistent modification, without committing ourselves as to its exact genetic basis.

Composition of Protozoan Species

All statistical studies that have been made on parasitic protozoa indicate that each species is a composite of diverse biotypes varying in mean size, comparable to the condition first shown by Jennings (1908) in *Paramecium.* Among these studies are those of Dobell and Jepps (1918) on *Endamoeba histolytica,* Hegner (1921) on *Trypanosoma diemyctyli,* Taliaferro (1921, 1923, and 1926) on *T. lewisi,* Hegner (1922) and Tsuchiya (1930) on *Giardia agilis* and *G. lamblia,* and Hall and Becker (1935) and Reyer (1937) on coccidia. The work by the senior author on *T. lewisi* was facilitated by the fact that all multiplication of this parasite is restricted to the first ten days of the infection in the rat, after which the trypanosomes exist in the blood as nonreproducing adults. Study of these adult forms indicated the following: The variability for total length in a population is very low (about 3 per cent). Growing the same pure line or clon in different rats sometimes results in small but significant differences in mean size. Passage of the clon through its invertebrate host (a flea) generally results in a significant increase in variability for total length, but in no greater differences in mean size than would be expected from the fact that the organisms were studied in different rats. Although the species, *T. lewisi,* is probably a composite of many biotypes, a single "wild" infection may represent a single biotype—a fact which may be explained by the severe selection of the infective forms and by the small margin of survival whenever they pass from the flea to the rat. Although some differences in size among strains of parasites are apparently due to environment, *i.e.,* the host, as indicated in this work on *T. lewisi,* all of them cannot be immediately due to environment, because they persist when several strains showing such differences are grown in the same host.

General observations and a few careful experimental studies (see review in Meleney and Frye 1933) indicate that parasitic species are also a composite of biotypes differing in pathogenicity. Thus, Meleney and Frye (1933, 1935) have shown that various strains of *Endamoeba histolytica* vary in pathogenicity. Some observers believe that the strain of *E. histolytica* that produces small cysts is relatively non-pathogenic; in other words, that non-pathogenicity in this species may be linked with size of cyst.

Strains composing various species of trypanosomes (review in Taliaferro 1929) and malarial organisms (review in Taliaferro and Mulligan 1937) vary markedly in their antigenic composition and in some cases show almost complete antigenic separateness. The investigations discussed later with regard to trypanosomes indicate that these differences in antigenic constitution may arise quickly from changes in environment.

Dauermodifikationen, or Environmentally Induced, Persistent Modifications

Such physiological characters as the virulence of protozoa for a given host are frequently modified by a change of host species, by rapid passage in the same species of host, or by cultivation on artificial media; but they have not been studied as extensively as have certain physiological characters modified by antibodies and drugs.

The acquisition of antibody resistance by the trypanosomes, with a concomitant change in antigenic composition, is an extremely interesting case of a character apparently environmentally induced which is inherited for many asexual generations. The short summary, presented here, is based on the work of Franke (1905), Rodet and Vallet (1906), Massaglia (1907), Ehrlich (1909), Ehrlich, Roehl, and Gulbransen (1909), Levaditi and Mutermilch (1909), Rosenthal (1913), and Ritz (1914, 1916). (These are reviewed by Taliaferro 1926a and 1929, and in part by Dobell 1912). Various pathogenic trypanosomes reproduce at a more or less constant rate in the blood of certain animals, such as the guinea-pig and dog, but the progeny, instead of increasing progressively, as they do in the mouse, show alternate decreases (crises) and increases (relapses) in number. The crises can be correlated with the production of trypanolysins in the blood by the host, and the relapses can be correlated with the acquisition of a resistance to the trypanolysin by the trypanosome. Not only is the relapse strain resistant to the antibody producing the previous crisis, but, as shown by cross-immunity experiments, the relapse strain is different antigenically from the parent strain. These studies and conclusions were made possible by the fact that the various relapse and parent strains can be extracted and maintained unchanged during asexual reproduction through hundreds of mouse passages, because antibodies are not formed against them in the mouse. Related to antibody resistance is the resistance of pathogenic trypanosomes to normal human serum, which was reported initially by Jacoby (1909).

Drug resistance was first discovered by Franke (1905) in Ehrlich's laboratory and has been extensively studied since. Briefly stated, when a given species of trypanosome is treated with repeated subcurative doses of a drug, the parasites may become resistant to doses larger than those ordinarily needed to cure. A particularly interesting type of drug resistance, because it seems to be associated with the inheritance of a morphological change, was first studied by Werbitzki (1910). He found that *Trypanosoma brucei* may lose its parabasal body when grown in mice treated with dyes belonging to the pyronin, acridin, and oxazin groups. These dyes all possess an orthoquinoid structure, according to Ehrlich (1909a). Laveran and Roudsky (1911, 1911a) presented evidence indicating that they have a special affinity for the parabasal body and that the organelle is actually destroyed by autoöxidation. The organisms without parabasal bodies are essentially normal in other respects. This persistent modification remains constant through many subinoculations in mice and seems similar to the condition in the naturally occurring, aparabasal species, *T. equinum*. It occasionally arises spontaneously (Wenyon 1928).

Probably the underlying mechanism involved in the formation of these various strains of resistant protozoa is the same and similar to the acquisition of resistance to various chemicals by free-living forms, such as extensively studied by Jollos (1921). We shall, therefore, consider them together. Ehrlich, Roehl, and Gulbransen (1909) and Levaditi and Mutermilch (1909) showed that antibody resistance can be induced by contact with immune serum *in vitro.* Levaditi and McIntosh (1910) found that most of the organisms are killed during this process but that the few that survive, when injected into mice, give rise to a resistant strain. The formation of both antibody- and chemo-resistant strains *in vivo* is also associated with the killing of a large proportion of the population. This led Levaditi and Mutermilch (1909a), Mutermilch and Salamon (1928), and others to postulate a process of true selection to explain the formation of antibody-resistant strains. In the process, susceptible strains were killed off. Recently, while studying acriflavine resistance in *Bodo caudatus*, an easily culturable free-living form similar in structure to trypanosomes, Robertson (1929) was impressed with the selection of "strains" within a clon, but believed that in addition there must be an actual change in the organism. Since the selection was within a clon, as was also Ritz's (1916) work on trypanosomes, it cannot be a selection of distinct biotypes, as ordinarily understood, from a mixed population, but it does suggest that changes in environment may cause some of the parasitic protozoa to split into diverse biotypes which are permanent enough to be acted upon by selection. Selection in these parasitic forms acts more rapidly than in free-living protozoa, as described by Jennings (1920) and others.

Rosenthal (1913) believed that antibody resistance results from contact of the trypanosomes with "relapse substances" in the serum which are different from the trypanolysins, but since such an hypothesis makes death of the organisms and consequent selection unessential, it is difficult to bring it into line with the results obtained with free-living forms.

Antibody and drug resistance are often inherited for countless asexual generations —sometimes for over 400 mouse passages. They are eventually lost, however, sometimes rapidly and sometimes gradually. Probably the quickest loss of resistance is seen when an antibody-resistant strain comes in contact with a new antibody. Under such circumstances it quickly gains a new antigenic composition and a resistance to the new antibody. These changes probably parallel the severity of environmental change, which would be in line with some of Jollos' (1921) work on *Paramecium.*

In addition to these general characteristics, it is interesting to note that Neuschlosz (1919, 1920) found that acquired resistance to a chemical in clons of *Paramecium* is associated with acquisition of a power to destroy the specific chemical or to convert it into a less poisonous compound, as in the conversion of a toxic trivalent arsenical into a less toxic pentavalent form.

Gonder (1911) thought that drug-resistant strains lost their resistance when passed through the invertebrate host, but Reichenow and Regendanz (1927), Duke (1927), and especially Yorke, Murgatroyd, and Hawking (1933, 1933a) found that arsenic resistance in trypanosomes survives passage through the invertebrate host. The latter authors found that *Trypanosoma brucei,* which can be made highly resistant to the aromatic arsenicals, can retain the resistance after two successive passages through *Glossina.* Another aspect of passing the parasite through the invertebrate host should be noted. The work by Taliaferro (1926) on *T. lewisi* indicates that a clon may split up into different biotypes in the invertebrate host. Such apparently new biotypes seem to be environmentally induced, just as are the antibody- and drug-resistant strains, since sexual phenomena are unknown in trypanosomes.

Although the sexual process, with its recombinations and shufflings of genetical elements, undoubtedly gives rise to new characteristics, it may be a great leveler of environmentally induced characters. Thus, in Jollos' (1921) work on *Paramecium,* most persistent modifications were lost after conjugation or endomixis. The very fact that the majority of the known, heritable modifications in parasitic protozoa which appear to be environmentally induced occur among trypanosomes may be associated with the fact that sexual phenomena are absent. Future work may well show that these organisms are either extremely impressionable to environmental effects or very subject to mutation, whereas such forms as *Plasmodium* are less affected because sexual processes normally occur at each new infection of the invertebrate host.

At the present time, it is impossible to decide whether persistent modifications are due to changes in gene constitution or, if they are not, whether they may eventually lead to true mutations. Jennings (1929) has pointed out the insecurity of Jollos' (1921) assumption that mutations or genic change in the micronucleus must be permanent, and of his theoretical conclusion that the *Dauermodifikationen* do not lie in the micronucleus but either in the cytoplasm or in the macronucleus. Recently Lindegren (1935), after a reexamination of Jollos' work, concludes that the *Dauermodifikationen* behave as genic rather than nongenic changes, and believes that the changes can be explained "as the result of gene mutations which were selected by an adverse environment, with reverse mutations to normal being selected upon return of the mutant to the normal environment." It would seem that further radical analysis may depend upon the development of methods to follow individual organisms in sexual crosses, such as Sonneborn and Lynch (1934) have done in *Paramecium*.

No matter what the true genetic and physiological bases may be for the modifications which appear to be environmentally induced, they are of extreme importance in overcoming the defensive processes of the host and in the eventual survival of these parasites. Thus, the acquisition of resistance to human serum may explain, at least in part, the power of *Trypanosoma gambiense* to survive in man. The acquisition of antibody resistance with its concomitant change in antigenic composition permits the parasite to survive repeated defensive processes of the host. The acquisition of drug resistance and its survival through the invertebrate host indicate the extent to which modifications apparently induced by the environment may persist under natural conditions and suggest that they may be permanent enough to be acted upon by natural selection and give rise to new varieties.

Finally, it should be emphasized that almost all physiological characters of the parasitic protozoa are more or less dependent upon their environment, *i.e.*, the living host, which, in turn, depends upon the genetics of the host. For this reason it is impossible to consider the physiology and characteristics of the parasite without considering at the same time the genetic constitution of the host.

References Cited

Dobell, C. 1912–1913. Some Recent Work on Mutation in Micro-organisms. *J. Genetics,* 2: 201.

Dobell, C. and Jepps, M. W. 1918. A Study of the Diverse Races of Entamoeba histolytica Distinguishable from One Another by the Dimensions of Their Cysts. *Parasit.,* 10: 320.

Duke, H. 1927. Experiments on the Cyclical Transmission of Glossina palpalis of Arsenic-fast Strains of Polymorphic Trypanosomes. *Interim Rept., League of Nations Intern. Comm. on Human Trypanosomiasis,* C.H. 536, Geneva. p. 23.

Ehrlich, P. 1907. Chemotherapeutische Trypanosomen-Studien. *Berlin. klin. Wochenschr.,* 44: 233, 280, 310, 341.

———. 1909. Ueber Partialfunktionen der Zelle. *Münch. med. Wochenschr.,* 56: 217.

———. 1909a. Ueber die neuesten Ergebnisse auf dem Gebiete der Trypanosomenforschung. *Arch. Schiffs. u. Tropenhyg.,* Beiheft 13: 321.

Ehrlich, P., Roehl, W. and Gulbransen, R. 1909. Ueber serumfeste Trypanosomenstämme; Bemerkungen zu der Arbeit von Levaditi und Mutermilch. *Zeitschr. f. Immunitätsf., Orig.,* 3: 296.

Franke, E. 1905. Therapeutische Versuche bei Trypanosomenerkrankung. Veterinär-med. Dissert., Giessen.

Gonder, R. 1911. Untersuchungen über arzneifeste Mikroorganismen. I. Trypanosoma lewisi. *Centralbl. f. Bakt., Orig.,* I, 61: 102.

Hall, P. R. and Becker, E. R. 1935. The Size of the Oöcyst in a Selected Strain of Coccidia. *Proc. Iowa Acad. Sci. for 1933,* 40: 235.

Hegner, R. W. 1921. Measurements of Trypanosoma diemyctyli from Different Hosts and Their Relation to Specific Identification, Heredity and Environment. *J. Parasit.,* 7: 105.

———. 1922. The Systematic Relationship of Giardia lamblia Stiles, 1915, from Man and Giardia agilis Kunstler, 1882, from the Tadpole. *Amer. J. Hyg.,* 2: 435.

Jacoby, M. 1909. Ueber Serumfestigkeit und die Einwirkung von menschlichem Blutserum auf Trypanosomen. *Zeitschr. f. Immunitätsf., Orig.*, 2: 689.

Jennings, H. S. 1908. Heredity, Variation and Evolution in Protozoa. *Proc. Amer. Phil. Soc.*, 47: 393.

————. 1920. Life and Death, Heredity and Evolution in Unicellular Organisms. Badger, Boston.

————. 1929. Genetics of the Protozoa. *Bibliogr. Genetica*, 5: 105.

Jollos, V. 1921. Experimentelle Protistenstudien. I. Untersuchungen über Variabilität und Vererbung bei Infusorien. *Arch. Protistenk.*, 43: 1.

Laveran, A. and Roudsky, D. 1911. Au sujet de l'action de l'oxazine (chlorure de triaminophénazoxonium) sur les trypanosomes. *Compt. rend. Acad. Sci.*, 153: 226.

————. 1911a. Au sujet de l'action de l'oxazine (chlorure de triaminophénazoxonium) et de l'akridine (diphénylméthane) sur les trypanosomes. *Compt. rend. Acad. Sci.*, 153: 916.

Levaditi, C. and Mutermilch, S. 1909. Recherches sur la méthode de Bordet et Gengou appliquée à l'étude des trypanosomiases. *Zeitschr. f. Immunitätsf., Orig.*, 2: 702.

————. 1909a. Le mécanisme de la création des variétés de trypanosomes résistant aux anticorps. *Compt. rend. Soc. biol.*, 67: 49.

Levaditi, C. and McIntosh, J. 1910. Mécanisme de la création de races de trypanosomes résistantes aux anticorps. *Bull. Soc. Path. Exot.*, 3: 368.

Lindegren, C. C. 1935. Genetical Studies of Bacteria. II. The Problem of Bacterial Variation. *Zentralbl. f. Bakt., Orig.*, II, 93: 114.

Massaglia, M. A. 1907. Des causes des crises trypanolytiques et des rechutes qui les suivent. *Compt. rend. Acad. Sci.*, 145: 687.

Meleney, H. E. and Frye, W. W. 1933. Studies of Endamoeba histolytica and Other Intestinal Protozoa in Tennessee. V. A Comparison of Five Strains of E. histolytica with Reference to Their Pathogenicity for Kittens. *Amer. J. Hyg.*, 17: 637.

————. 1935. Studies of Endamoeba histolytica and Other Intestinal Protozoa in Tennessee. IX. Further Observations on the Pathogenicity of Certain Strains of E. histolytica for Kittens. *Amer. J. Hyg.*, 21: 422.

Mutermilch, S. and Salamon, E. 1928. Contribution à l'étude du mécanisme de la création des races des trypanosomes du nagana anticorpsrésistantes. *Compt. rend. Soc. biol.*, 98: 345.

Neuschlosz, S. M. 1919. Untersuchungen über die Gewöhnung an Gifte. I. Mitteilung: Das Wesen der Chininfestigkeit bei Protozoen. *Pflüger's Arch. ges. Physiol.*, 176: 223.

————. 1920. Untersuchungen über die Gewöhnung an Gifte. II. Die Festigkeit der Protozoen gegen Farbstoffe. III. Das Wesen der Festigung von Protozoen gegen Arsen und Antimon. *Pflüger's Arch. ges. Physiol.*, 178: 61, 69.

Reichenow, E. and Regendanz, P. 1927. Ueber die Flohpassage normaler und mit Arsenophenylglycin vorbehandelter Rattentrypanosomen. *Abh. a. d. Geb. d. Auslandskunde, Hamburgische Univ.*, 26: 446.

Reyer, W. 1937. Biometrische Untersuchungen an Cysten von Barrouxia schneideri aus dem Darm von Lithobius forficatus. *Arch. Protistenk.*, 88: 431.

Ritz, H. 1914. Ueber Rezidive bei experimenteller Trypanosomiasis. *Deutsch. med. Wochenschr.*, 40: 1355.

————. 1916. Über Rezidive bei experimenteller Trypanosomiasis. *Arch. Schiffs. u. Tropenhyg.*, 20: 397.

Robertson, M. 1929. The action of Acriflavine upon Bodo caudatus. *Parasit.*, 21: 375.

Rodet, A. and Vallet, G. 1906. Contribution à l'étude des trypanosomiases. *Arch. Méd. expér. et Anat. path.*, 18: 450.

Rosenthal, F. 1913. Untersuchungen über die Genese des Rezidive bei der experimentellen Trypanosomeninfektion. *Zeitschr. Hyg. u. Infektionskr.*, 74: 489.

Sonneborn, T. M. and Lynch, R. S. 1934. Hybridization and Segregation in Paramecium aurelia. *J. Exp. Zool.*, 67: 1.

Taliaferro, W. H. 1921. Variation and Inheritance in Size in Trypanosoma lewisi. *Proc. Nat. Acad. Sci.*, 7: 138, 163.

————. 1923. A Study of Size and Variability, Throughout the Course of "Pure Line" Infections, with Trypanosoma lewisi. *J. Exp. Zool.*, 37: 127.

————. 1926. Variability and Inheritance of Size in Trypanosoma lewisi. *J. Exp. Zool.*, 43: 429.

————. 1926a. Host Resistance and Types of Infections in Trypanosomiasis and Malaria. *Quart. Rev. Biol.*, 1: 246.

————. 1929. The Immunology of Parasitic Infections. Century Co., New York.

Taliaferro, W. H. and Mulligan, H. W. 1937. The Histopathology of Malaria with Special Reference to the Function and Origin of the Macrophages in Defence. *Ind. Med. Res. Mem.*, 29: 1.

Tsuchiya, H. 1930. A Comparative Study of Two Diverse Strains of Giardia lamblia Stiles, 1915. *Amer. J. Hyg.*, 12: 467.

Wenyon, C. M. 1928. The Loss of the Parabasal Body in Trypanosomes. *Trans. Roy. Soc. Trop. Med. and Hyg.*, 22: 85.

Werbitzki, F. W. 1910. Ueber blepharoblastlose Trypanosomen. *Centralbl. f. Bakt., Orig.*, I, 53: 303.

Yorke, W., Murgatroyd, F. and Hawking, F. 1933. Effect of Passage through Glossina on the Resistance of a Tryparsamide-fast Trypanosome. *Brit. Med. J.*, 1: 176.

————. 1933a. Studies in Chemotherapy. X. Further Observations on the Transmissibility of Tryparsamide-resistance by Glossina. *Ann. Trop. Med. and Parasit.*, 27: 157.

THE INFLUENCE OF HOST CONSTITUTION ON THE PARASITE

By CLAY G. HUFF

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY, THE UNIVERSITY OF CHICAGO, CHICAGO, ILL.

Although several excellent reviews of investigations on the influence of host constitution on parasites have been published in recent years (Kozelka 1929; Link 1932; Lambert 1933; Gowen 1933, 1935; Hill 1934; and Turpin 1936), it is believed that certain phases especially pertinent to the interests of this symposium should be emphasized. It is, therefore, my desire to summarize our knowledge of the importance of the hereditary constitution of the host in relations between host and parasite and to point out the special importance of heredity in animal parasitology. ''Pathogenicity'' is a term used to denote the severity of the effect of one organism, the parasite, upon another organism, the host. It follows that a consideration of the genetics of the parasite is incomplete without consideration of the genetics of the host, and would not fulfill the purpose of a symposium of this kind.

It is not surprising that a principle so universal as the effect of inheritance on susceptibility or resistance to disease should have been recognized for a long time. We have in such words as ''constitution'' and ''diathesis,'' and in such adages as ''What is one man's meat is another man's poison,'' evidence of a widely prevalent belief that inherent makeup plays some part in susceptibility to disease. Such beliefs have been held by some scientists and physicians from the time of Hippocrates. They received less attention, however, after the beginning of the bacteriological era with its emphasis upon *the* etiological agents of disease. ''Constitution'' and ''diathesis'' fell into disrepute at that time and the ''causes'' of disease came to mean principally those things that were grown in test tubes.

The scientific study of inheritance of factors influencing susceptibility to disease began after our knowledge of the laws of heredity had been put on a secure basis. The past decade has seen a rapid development. Indirect studies made by Pearson in 1907, followed by others at somewhat later dates, showed a higher correlation between parent and offspring than between husband and wife, and thus indicated the importance of the patient's heredity in the incidence of tuberculosis, but it was not until 1917 that Tyzzer presented experimental evidence of inheritance of susceptibility to a bacterial infection. He worked with *Bacillus piliformis* and its effects upon Japanese waltzing mice and common white mice.

One of the first experiments of this kind to yield quantitative results of value to modern genetics might be called a natural experiment, inasmuch as it occurred without being planned. Hagedoorn-Labrand and Hagedoorn (1920) were studying the inheritance of weight by using an oriental waltzing mouse which hybridized easily with the common white mouse. At a time when a number of mice were on hand, including the parent stocks and various hybrids, an epizootic of staphylococcus infection caused the death of all the waltzing mice, whereas none of the white mice succumbed. The F_1 hybrids all survived; of the F_2 hybrids, 91 lived and 34 died. Furthermore, in the backcross of the F_1 to waltzing mice, 25 lived and 32 died, whereas in the backcross of the F_1 to white mice, 50 lived and 1 died.

The first planned experiments upon inheritance and resistance were carried out by Wright and Lewis (1921) who tested differences in resistance to tuberculosis among families of inbred guinea pigs. They showed that factors determining resistance to tuberculosis in a family were not closely related to other elements of vigor, including rate of growth, adult weight, size of litter, percentage of young born alive, and percentage of young raised to weaning. Lewis and Loomis (1928) supplemented these studies by testing the capacity of guinea pigs to produce antibodies and relating it to familial resistance to tuberculosis. Finding imperfect but suggestive correlations between ''allergic irritability'' and differences in the resistance of the same families to tuberculosis induced by inoculation, as determined by Wright and Lewis (1921), they suggested that ''allergic irritability'' is one of the several heritable characters responsible for natural immunity to tuberculosis.

These earlier papers tend to illustrate the type of work that has yielded such valuable information on this subject. Lack of time prevents more than mention of recent work, but the following list indicates the wide variety of hosts and of infectious agents studied:

Bacterial diseases of mice and rats

Webster 1924; Lesné and Dreyfus-Sée 1928; Irwin 1929; Schott 1932; Gowen and Schott 1933.

Bacterial diseases of rabbits

Manresa 1932.

Bacterial diseases of fowl

Frateur 1924; Roberts and Card 1926–1927; Lambert 1932.

Filterable viruses

Of maize, in leafhoppers, Storey 1932; of yellow fever, in mice, Lynch and Hughes 1936.

Protozoa

Malaria in mosquito, Huff 1929; coccidia in rats, Becker 1933.

Worms

Cysticercus—sarcoma of rats, Curtis, Dunning, and Bullock 1933.

The hosts range from mosquitoes and leafhoppers to rats, guinea pigs, and fowl. The infectious agents include filterable viruses, bacteria, protozoa, and worms. In all cases, the authors believed they had evidence that heredity played some part in susceptibility to the infectious agent, and in most cases there was good genetical evidence suggesting the manner in which the inheritance occurred. I have been unable as yet to find an account of any study showing that the heredity of the host plays no part in susceptibility or resistance to infection.

My work with avian malarial parasites and their invertebrate hosts, the culicine mosquitoes, appears to have special interest in this connection. These parasites are pathogenic for their avian hosts, but there is no clear evidence that they have any harmful effects on the mosquitoes which they infect. Any study of the genetics of these parasites in the mosquito has the possible advantage that their pathogenicity can be studied in the avian host, while their other biological characteristics can be studied in the mosquito under better controlled conditions of temperature and length of infection. In the preceding paper Dr. Taliaferro has mentioned some of the obstacles to genetical studies upon such parasitic forms as malarial parasites, which cannot yet be successfully cultivated outside the cells of the host. It has been in recognition of such obstacles that an attempt has been made to study the inheritance of both parasite and mosquito simultaneously insofar as this is possible.

The asexual cycle of these parasites (*Plasmodium cathemerium* and *P. relictum* have been the most studied) occurs in the blood cells of the canary. It can be maintained for long periods of time by passing infected blood into new birds at intervals. Since the sexual cycle cannot occur except in the mosquito, it should be possible to separate our study of asexual changes from the effects of sexual reproduction. Thus, one of our strains of *P. relictum,* isolated 26 years ago from a sparrow, has been maintained in canaries since that time without the intervention of sexual reproduction, and we have good evidence that the parasites have been undergoing asexual reproduction at the rate of one generation every 24 hours during that long period. At any time, however, this strain may be allowed to undergo sexual reproduction by permitting a mosquito to transmit it from one canary to another.

Selection experiments performed upon mosquitoes examined for the percentage of infected individuals in each generation showed (Huff 1929) rapidly increasing percentages of susceptibles in the offspring from susceptible females, while percentages of susceptibles in progenies of uninfected females rapidly decreased. It was possible (Huff 1931) to demonstrate that susceptibility of the mosquito, *Culex pipiens,* to *Plasmodium cathemerium* behaves as a Mendelian recessive character. Moreover, statistical studies have revealed a high correlation between numbers of parasites surviving in the stomachs of susceptible mosquitoes from two blood meals spaced in such a way that two broods can be identified at dissection. There seems to be no other possible conclusion than that the degree of susceptibility of the individual is an inherent characteristic. If this be so, we may eventually be able to analyze the manner in which it is inherited.

In recent unpublished work upon size and growth of these parasites in mosquitoes kept at constant temperatures, significant differences in oocyst size have been found in the parasites of different individual mosquitoes. The question is as yet unsettled as to whether this is due to differences in the suitabilities of the mosquitoes for growth of the parasites or to differential selection by the mosquito of certain components of a diverse population of parasites varying in their rates of growth. I mention the problem here since it seems to be one of the best examples of the manner in which the genetics of the host is so intimately associated with the genetics of the parasite as to make futile the study of one alone.

I have tried in this discussion to show the importance of investigation of host genetics for an understanding of the genetics of pathogenic organisms in general. I should like now to point out its importance in my own field, parasitology. Many animal parasites of man and animals are obligate parasites, or parasites that can be cultivated upon artificial media only indifferently well. This makes it necessary to study them within their hosts. In the type of experiment which requires a fairly constant environment, we cannot ignore the possibility that individual hosts may vary so widely in their constitutions as to introduce errors due to different environments. In most work with pathogenic organisms we are concerned with the effects of rate of passage through hosts. Many experiments cannot be carried out on enough diverse strains of animals to make the results significant in spite of the diverse heredities of these strains. There is the alternative course, however, that pedigreed animals whose reactions to the parasites have been studied can be used in some experiments.

Parasitologists are interested not only in parasites that cause harmful effects directly but also in another group of organisms which bring harm indirectly to their host, namely, the arthropods which transmit disease. Of the vast number of insects and other arthropods which bite man or animals or otherwise come into contact with them so as to make disease transmission possible, only a small percentage are important vectors of disease. These owe their capacity for transmitting the agents of disease to certain definite biological characteristics, such as tropisms towards their hosts or food or waste products of the latter, hibernating habits, breeding habits, and susceptibilities to the organisms to be transmitted. All such characteristics are variable and some of them are known to be modified through selection. It will be apparent, therefore, that we shall need to know more about their heredity before we can understand fully the relation of arthropods to disease transmission.

In conclusion, I wish to state that it has not been my intention, by emphasizing the importance of a knowledge of the genetics of the host, to minimize by implication the great importance of the genetics of parasites, the main subject of this symposium, but rather to point out how the two problems are so related as to be better pursued together than apart.

References Cited

Becker, E. R. and Hall, P. R. 1933. The Possible Rôle of Inheritance in the Quantitative Character of a Coccidian Infection of the Rat. *Parasit.*, 25: 397.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. 1933. Genetic Factors in Relation to the Etiology of Malignant Tumors. *Amer. J. Cancer,* 17: 894.

Frateur, J. L. 1924. The Hereditary Resistance of the Fowl to the Bacillus of Diphtheria. *Proc. World's Poultry Congr.*, 2: 68.

Gowen, J. W. 1933. On the Genetic Structure of Inherited Constitution for Disease Resistance. *Quart. Rev. Biol.*, 8: 338.

————. 1935. Genetic Constitution as a Factor in Disease. *Sigma Xi Quart.*, 23: 103.

Gowen, J. W. and Schott, R. G. 1933. Genetic Constitution in Mice as Differentiated by Two Diseases, Pseudorabies and Mouse Typhoid. *Amer. J. Hyg.*, 18: 674.

Hagedoorn-Labrand, A. C. and Hagedoorn, A. L. 1920. Inherited Predisposition for a Bacterial Disease. *Amer. Nat.*, 54: 368.

Hill, A. B. 1934. The Inheritance of Resistance to Bacterial Infection in Animal Species. *Med. Res. Council Sp. Rept.*, No. 196.

Huff, C. G. 1929. The Effects of Selection upon Susceptibility to Bird Malaria in Culex pipiens L. *Ann. Trop. Med. & Parasit.*, 23: 427.

————. 1931. The Inheritance of Natural Immunity to Plasmodium cathemerium in Two Species of Culex. *J. Prev. Med.*, 5: 249.

————. 1934. Comparative Studies on Susceptible and Insusceptible Culex pipiens in Relation to Infections with Plasmodium cathemerium and P. relictum. *Amer. J. Hyg.*, 19: 123.

Irwin, M. R. 1929. The Inheritance of Resistance to the Danysz Bacillus (S. enteritidis) in the Rat. *Genetics,* 14: 337.

Kozelka, A. W. 1929. The Inheritance of Natural Immunity among Animals. *J. Hered.*, 20: 519.

Lambert, W. V. 1932. Natural Resistance to Disease in the Chicken. *J. Immunol.*, 23: 229.

————. 1933. The Evidence for Inheritance of Resistance to Bacterial Diseases in Animals. *Quart. Rev. Biol.*, 8: 331.

Lesné, E. and Dreyfus-Sée, G. 1928. Sélection d'espèces animales à caractères immunitaires fixes. Transmission de ces caractères selon les lois mendéliennes et modifications durables obtenues par les vaccinations répétées. *Compt. rend. Soc. biol.*, 98: 922.

Lewis, P. A. and Loomis, D. 1928. Ulcerative Types as Determined by Inheritance and as Related to Natural Resistance against Tuberculosis: An Experimental Study on Inbred Guinea Pigs. *J. Exp. Med.*, 47: 449.

Link, G. K. K. 1932. The Rôle of Genetics in Etiological Pathology. *Quart. Rev. Biol.*, 7: 127.

Lynch, C. J. and Hughes, T. P. 1936. The Inheritance of Susceptibility to Yellow Fever Encephalitis in Mice. *Genetics,* 21: 104.

Manresa, M. 1932. Inheritance of Resistance and Susceptibility to Infectious Abortion. *J. Infect. Dis.*, 51: 30.

Pearson, K. 1907. A First Study of the Statistics of Pulmonary Tuberculosis. *Drapers' Co. Res. Mem.*, No. 2.

Roberts, E. and Card, L. E. 1926. The Inheritance of Resistance to Bacillary White Diarrhea. *Poultry Sci.*, 6: 18.

Schott, R. G. 1932. The Inheritance of Resistance to Salmonella aertrycke in Various Strains of Mice. *Genetics,* 17: 203.

Storey, H. H. 1932. The Inheritance by an Insect Vector of the Ability to Transmit a Plant Virus. *Proc. Roy. Soc. London, B,* 112: 46.

Turpin, R. 1936. De l'influence des qualités héréditaires sur la sensibilité des animaux à l'égard des maladies infectieuses. *Rev. d'Immunol.*, 1936: 54.

Tyzzer, E. E. 1917. A Fatal Disease of the Japanese Waltzing Mouse Caused by a Spore-bearing Bacillus (Bacillus piliformis N. SP.). *J. Med. Res.*, 37: 307.

Webster, L. T. 1924. Microbic Virulence and Host Susceptibility in Paratyphoid-enteritidis Infection of White Mice. III. The Immunity of a Surviving Population. IV. The Effect of Selective Breeding on Host Resistance. *J. Exp. Med.*, 39: 129, 879.

Wright, S. and Lewis, P. A. 1921. Factors in the Resistance of Guinea-pigs to Tuberculosis, with Especial Regard to Inbreeding and Heredity. *Amer. Nat.*, 55: 20.

THE ORIGIN OF PHYSIOLOGIC RACES OF RUST FUNGI THROUGH HYBRIDIZATION[1]

By J. H. CRAIGIE

DOMINION RUST RESEARCH LABORATORY, WINNIPEG, MANITOBA, CANADA

INTRODUCTION

THE rust fungi comprise a very large group of obligate parasites which attack many unrelated groups of plants. Certain of them, such as the different cereal rusts, white pine blister rust, as well as others, are, on account of their destructiveness, of great economic importance. None, however, has been responsible for greater damage than stem rust of cereals (*Puccinia graminis* Pers.). It is largely with it that the present paper deals, for investigations on the origin of physiologic races in the rust fungi have been largely confined to it.

Stem rust attacks wheat, barley, oats, rye, and a number of grasses. Prior to 1894, it was generally regarded as a simple species, but in that year Eriksson (1894) showed that stem rust comprises several distinct varieties which can be readily differentiated by the hosts that they can attack. Morphologically, however, the varieties are practically indistinguishable. They are commonly designated as wheat stem rust, oat stem rust, rye stem rust, etc., according to their principal cereal or grass host. In 1917 and subsequently, Stakman and his coworkers (1917, 1919, 1922) showed that these varieties, or at least those affecting cereals, comprise a number of different races, which likewise are distinguishable by their infective ability on a selected group of wheat, oats, or rye varieties, according to the variety of rust concerned.

Until this host specialization of stem rust was fully elucidated, a great deal of confusion existed relative to the susceptibility or resistance of different cereal varieties. Obviously under such conditions, little progress could be made in breeding for stem-rust resistance. Afterwards, however, plant breeding could proceed with greater assurance, but always this assurance was tempered by the uncertainty as to whether or not some race of stem rust might appear that would possess greater infective ability than any races already known. From time to time new races were being discovered, and the question naturally arose as to the origin of rust races and as to the possibility of new races arising that would attack severely new cereal varieties that were showing promise of high rust resistance.

The possibility of stem-rust races hybridizing on barberry, the alternate host of the fungus, and giving rise to new races was suggested by Stakman, Piemeisel, and Levine (1918) as early as 1918. Through the work of Craigie (1927a, 1928), this possibility became a demonstrable reality. He showed that the stem-rust fungus is heterothallic, the sexual stage or its equivalent occurring on barberry, and he developed a technique by which crosses could be made between varieties or races of stem rust. Since that time, considerable attention has been given to a study of crosses between such varieties and races, and of selfed lines of individual races, with a view to elucidating the origin of races and the manner in which pathogenicity is inherited.

Hybridization might be expected to occur in heterothallic rusts that comprise physiologic races. Heterothallism and physiological specialization, however, have been investigated in relatively few rusts, and only in two, as far as the author is aware, have crosses between races actually been made. A cross between two races of leaf rust of wheat (*Puccinia triticina* Erikss.) was made by Waterhouse (1932) in Australia, and from it he obtained two races that were new to that country. All the other crosses have been made between varieties and races of stem rust (*P. graminis*). Besides these two rusts, the following are known to be heterothallic: *Puccinia helianthi* Schw. (Craigie 1927), *Uromyces appendiculatus* (Pers.) Fries and *U. vignae* Barclay (Andrus 1931), *Puccinia coronata* Corda (Allen 1932a), *Gymnosporangium juniperi-virginianae* Schw. and *G. globosum* Farl. (Millar 1932), *P. sorghi* Schw. (Allen 1934a; Cummins 1931; Mains 1934), *Melampsora lini* (Pers.) Lév. (Allen 1934), *Gymnosporangium haraeanum* Syd. (Kawamura 1934), *P. phragmites* (Schum.) Körn. (Lamb 1935), *Cronartium ribicola* J. C. Fischer (Pierson 1933), and *U. graminis* (Niessl) Diet. (D'Oliveira 1938). In four of the twelve rusts just named, physiologic races occur. Apparently, in the others physiological specialization has not been investigated, but probably when this is done physiologic races will be found to occur in them also. It would seem likely that the possibility of hybridization exists in these twelve rusts at least, and, indeed, perhaps in many others.

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Hybridization between Varieties of Stem Rust

A great many crosses have been made between varieties of stem rust at the University of Minnesota (Cotter and Levine 1938; Levine and Cotter 1931; Levine, Cotter, and Stakman 1934; Stakman, Levine, and Cotter 1930) and some at the Dominion Rust Research Laboratory, Winnipeg (Johnson, Newton, and Brown 1932; Johnson and Newton 1933). Detailed information concerning the F_2 and subsequent progeny of these crosses is not yet available, and hence little can be said as to the inheritance of pathogenicity in such crosses. All that can be done here is to indicate in a general way the results obtained in the first generation. The data relative to these crosses are presented in Tables I, II, and III.

TABLE I

Physiologic Races Obtained from Reciprocal Crosses on *Berberis vulgaris* of *Puccinia graminis tritici* with *P. graminis agrostidis* and *P. graminis secalis*, Respectively
(Stakman, Levine, and Cotter, 1930)

Parents	F_1 generation
Tritici 36 × *Agrostidis*	*Tritici* 36, 61, 21, 17
Agrostidis × *Tritici* 36	No aecia formed
Tritici 36 × *Agrostidis*	*Tritici* 32
Agrostidis × *Tritici* 36	No aecia formed
Tritici 36 × *Agrostidis*	*Tritici* 36, **69**,* **67**, 52, 10, 38, 58, 61, **72**
Tritici 36 × *Secalis* 11	*Tritici* 36, 15
Secalis 11 × *Tritici* 36	*Secalis* 9, *Tritici* 57
“ 11 × “ 36	“ 11
Tritici 36 × *Secalis* 11	*Tritici* 32
Secalis 11 × *Tritici* 34	*Secalis* 11
“ 11 × “ 36	“ 11
“ 11 × “ 36	*Tritici* 36
“ 11 × “ 36	“ 21, **70**, **71**
Tritici 36 × *Secalis* 11	“ 67

* New races are indicated by numbers in bold face.

These results show conclusively that there is partial interfertility between varieties of stem rust and that, as a result of experimentally-made crosses, true hybrids have been produced. Some of these are new to science. Certain of them exhibit pathogenic characteristics resembling more or less closely those of one or the other parental variety, while others are definitely intermediate in pathogenicity between the two. Still others are far enough removed in pathogenic capabilities from either of the parent varieties to represent a different variety. For instance, a cross between the *poae* variety and the *agrostidis* variety (Table II) yielded races of the *tritici* variety, although neither of the parent varieties attack wheat. Or, again, the *hordei* variety, which in recent years has not been recognized as a variety, must now be revived to accommodate certain of the new hybrid races. There seems to be a definite tendency for crosses between the *tritici* and the *secalis* varieties to

TABLE II

Result of Crosses Made on *Berberis vulgaris* Between Varieties of *Puccinia graminis* (University of Minnesota)

Parents	F_1 generation
Tritici × *Secalis*	*Hordei variety*
(Levine and Cotter, 1931)	
Secalis 7 × *Tritici* 21	*Hordei variety*
" × " 101	
Tritici 34 × *Agrostidis*	
(Levine, Cotter, and Stakman, 1934)	
Tritici × *Agrostidis*	16 *Tritici*, 2 *Hordei*
Agrostidis × *Tritici*	5 *Tritici*, 2 *Hordei*, 1 *Secalis*
Secalis × *Agrostidis*	9 *Tritici*, 4 *Secalis*, 1 *Avenae*
Agrostidis × *Secalis*	4 *Tritici*, 1 *Secalis*
Poae × *Agrostidis*	3 "
Attempted crosses = 382, pustules producing aecia = 35% (Cotter and Levine, 1938)	

TABLE III

Results of Crosses Made on *Berberis vulgaris* Between Varieties of *Puccinia graminis* (Dominion Rust Research Laboratory)

Parents	F_1 generation
Tritici 95 × *Secalis*	*Tritici* **104*** and **111**
" 30 × "	" **70**
" 30 × "	" **112**
Secalis × *Tritici*	*Secalis*
Tritici 9 × *Agrostidis*	*Hordei* (?)
Agrostidis × *Tritici* 9	*Agrostidis*
(Johnson, Newton, and Brown, 1932)	
Tritici 9 × *Avenae* 6	(Hybrid belonging to no variety)
" 11 × " 6	(No aecia developed)
" 36 × " 6	
Avenae 6 × *Tritici* 9	(Aecia formed only in one pustule—no infection on wheat or oats)
" 6 × " 11	
" 6 × " 36	
(Johnson and Newton, 1933)	

* New races are indicated by numbers in bold face.

produce races of the *hordei* variety. From a cross between the *tritici* and *avenae* varieties, a hybrid was obtained that conforms to no known variety. It possesses the ability to infect normally certain varieties of wheat and barley as well as certain oat varieties and some grasses that are natural hosts of oat stem rust.

Hybridization between Races of Wheat Stem Rust

The first results on crosses between races of wheat stem rust were reported by Waterhouse (1929). From such a cross, there arose two races of this rust that were previously unknown in Australia.

During the past eleven years, a large number of crosses have been made between different races of wheat stem rust at the Dominion Rust Research Laboratory, Winnipeg (Johnson, Newton, and Brown 1934; Newton, Johnson, and Brown 1930, 1930a). Out of these crosses there have arisen approximately two dozen new races of this rust. These investigations have shown that most of the races collected in nature are heterozygous for pathogenicity, and that interfertility between the different races is complete. In a number of these crosses, the inheritance of pathogenicity was studied, in some of the progeny as far as the fourth and even the fifth generations. The occurrence of several mutant strains of abnormal spore color has afforded an opportunity for the study of the inheritance of urediospore color, but as these studies lie somewhat outside the scope of this symposium, it need only be remarked that urediospore color and pathogenicity are inherited independently, and that the inheritance of urediospore color conforms in general to Mendelian laws.

In regard to studies on the inheritance of pathogenicity, it should be pointed out that they involve the identification of the races represented in the F_1 and subsequent generations. Races of wheat stem rust are differentiated by the infection, or pustule, types that they produce on 12 standard varieties of wheat. By reason of the laborious technique incident to the identification of these races, the number of progeny cultures that can be studied is much more limited than if the character studied were readily discernible by visual inspection.

The results obtained relative to the inheritance of pathogenicity may be briefly summarized. In the main, they point to a Mendelian manner of inheritance.

First-generation Hybrids. Crosses between two races homozygous for pathogenicity yield F_1 hybrids that are identical in pathogenicity. On the other hand, if two heterozygous races are crossed, it frequently happens that different crosses between the same two races give rise to pathogenically different hybrids. A similar result is obtained when one of the parents is homozygous and the other heterozygous.

The F_1 hybrid of a cross between two races may be pathogenically identical with one or the other of the parents. For example, when Race 9 is crossed with Race 15 or with Race 52, the F_1 hybrid is identical in pathogenicity with the Race 9 parent. Apparently, then, in crosses between some races of wheat stem rust, the Mendelian principle of dominance is evident. On the other hand, the F_1 hybrids produced by crossing other races may be, and as a rule are, different in pathogenicity from the parents. Usually they show, at least on some of the differential varieties, a certain degree of intermediacy between the parents. In crosses between heterozygous races, however, F_1 hybrids occasionally arise that are of greater virulence on some wheat varieties than are the parental races.

Here it may be remarked that identity in pathogenicity does not necessarily imply absolute genetic identity in respect to pathogenicity. The twelve differential wheat varieties used to differentiate races of wheat stem rust are inadequate to resolve the rust into all its genotypically different strains. For example, in the cross Race 9 × Race 15, mentioned above, the parent Race 9 is homozygous for pathogenicity, but the F_1 hybrid, identified as Race 9, produced when selfed a progeny consisting of three other races in addition to Race 9. It is, therefore, genotypically different from the parent Race 9, but the differential varieties are incapable of giving expression to the difference.

Second-generation Hybrids. When the F_1 hybrids are selfed, the parental races are not infrequently recovered in the F_2 progeny, together with other races that show some of the pathogenic characteristics of the parents. Hence, there is evidently a segregation and recombination of factors governing pathogenicity. The hybrid race, or rather its phenotype, occurs more frequently than any other race.

The cross Race 9 × Race 36 may be taken as an example. The F_1 hybrid was identified as Race 17. In the second generation 126 cultures were studied and the F_2 progeny were resolved into one or another of seven different races. Race 17 occurred much more frequently than any of the other six. The F_2 population studied was too small to permit of the establishment of a factorial basis for the inheritance of pathogenicity; but there appears to be little doubt that the inheritance obeys Mendelian laws. The distribution of the cultures among the seven races is given in Table IV.

TABLE IV

A Comparison Between Observed Frequencies of Physiologic Races in the Second Hybrid Generation of the Cross Race 9 × Race 36, and Frequencies Calculated on the Basis of a 27: 9: 9: 9: 3: 3: 3: 1 Ratio

Physiologic races	17	1	36	11	15	85	57	
Observed frequencies	69	22	14	15	1	4	1	0
Calculated frequencies	54	18	18	18	6	6	6	2

In some of the crosses, certain races of the F_2 progeny are homozygous for pathogenicity, although the majority of them are heterozygous, as would be expected if Mendelian laws were operative. There is also evidence that a continued selfing of the progeny of a cross tends towards homozygosity in the races of later generations. Thus, the selfing of F_2 races generally results in fewer races than does the selfing of the F_1 hybrids. This tendency is illustrated in Fig. 1.

Maternal Cytoplasmic Inheritance

Although in crosses between some races of wheat stem rust the inheritance of pathogenicity seems rather definitely to be determined by Mendelian laws, in crosses between certain other races conformity to these laws is partially lacking. If pathogenicity is governed by Mendelian factors, it would be expected that, when a cross and its reciprocal are made between two races, one and the same race would emerge from both sides of the cross, provided, of course, that in the crossing a single pustule of each race was used. It has been found that, in crosses between certain races, the F_1 hybrid arising from one side of the cross differs somewhat in pathogenicity from the hybrid arising from the other side. In other words, some influence other than nuclear is operative. This influence is perpetuated in the second and subsequent generations, and has been attributed to the cytoplasm of the maternal parent (Johnson, Newton, and Brown 1934; Newton, Johnson, and Brown 1930a).

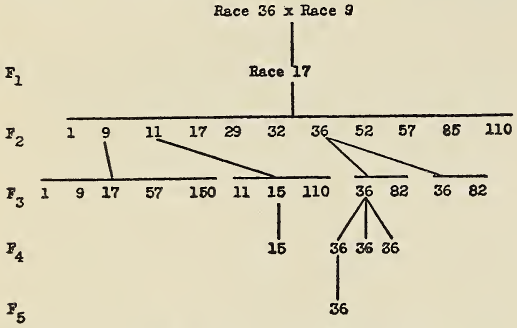

Fig. 1. Races Isolated in Different Generations of the Cross Race 36 × Race 9.

To illustrate this type of inheritance, the cross Race 36 × Race 14 may be taken. These two races differ widely in their ability to attack Marquis wheat, one of the differential varieties. Race 36 is highly pathogenic to Marquis, but Race 14 only weakly so. The F_1 hybrid from the Race 36 side of the cross was identified as Race 88, a race only slightly less pathogenic than Race 36 to Marquis. In the subsequent progeny of Race 88, at least as far as the fourth generation, all the races studied exhibited on Marquis the same infection type as Race 88. The F_1 hybrid from the reciprocal cross was weakly pathogenic to Marquis, as were also all races in subsequent progeny derived from it. If Mendelian factors alone were operative, some of the F_2 and subsequent progeny should have resembled in their infection type on Marquis the weak pathogenicity of the Race 14 parent. On the other eleven differential varieties, the inheritance seemed to be completely Mendelian.

Inbreeding of Stem Rust Races

In the course of these studies (Johnson and Newton 1938), attention was given to the origin of abnormal characteristics arising as a result of successive selfing, or inbreeding, of races of wheat stem rust. It was found that, when certain races were inbred, they gave rise to strains exhibiting one or more of the following abnormal characteristics: (*a*) aberrant urediospore color, (*b*) a decrease in vigor of sporulation, (*c*) a decrease in pathogenic vigor, (*d*) a greater sensitivity to high temperatures than races collected in the field, (*e*) loss of ability to produce aecia on barberry, and (*f*) the development of uredia and telia on barberry. Other races, however, when inbred, produced progeny that appeared normal in all respects.

Such abnormalities are rarely, if ever, met with in nature—probably for several reasons. In the first place, most of the abnormalities are detrimental to the strains of stem rust possessing them, and, consequently, such strains very probably have less chance of survival than normal strains. Besides, the abnormalities appear to be governed by recessive factors, which only find expression when brought together in a homozygous condition. In the laboratory, this homozygosity comes about as a result of inbreeding. In nature, however, crossing perhaps occurs just about as frequently as selfing, and inbreeding for several successive generations probably rarely occurs. Hence, there is perhaps little opportunity for the segregation of such factors. When abnormal races are crossed with normal ones, the abnormalities tend to disappear. Hybridization, therefore, tends to maintain the normal characteristics of the races. For these reasons, it would appear that the chances of abnormal races occurring in nature are rather limited.

EVIDENCE OF HYBRIDIZATION IN NATURE

That hybridization between races of stem rust occurs in nature can scarcely be doubted, although most of the evidence is indirect, as of necessity most such evidence must be. It has already been mentioned that the majority of races of wheat stem rust collected in the field are heterozygous for pathogenicity—a fact that may be taken as indicative of their hybrid origin. Stakman, Levine, Cotter, and Hines (1934) showed that, from 94 aecial collections, a different race was isolated for every four collections; while, from about 8,000 uredial collections, a different race was isolated for every 100 collections. From oats growing in the vicinity of barberry, Cotter (1932) isolated a new race of oat stem rust that very evidently originated either by the hybridization of two races or by the selfing of a race on barberry. Waterhouse (1929) has called attention to the great number of wheat stem rust races in the northern Mississippi Valley, where barberry readily becomes infected, and to the very small number of races of this rust in Australia, where barberry seldom rusts. There is little question that, through segregation and recombination of pathogenicity factors, new races of stem rust arise in nature through the medium of barberry, and that, in the process, hybridization of existing races plays an important part.

REFERENCE CITED

ALLEN, RUTH F. 1932. A Cytological Study of Heterothallism in Puccinia triticina. *J. Agr. Res.*, 44: 733.

————. 1932a. A Cytological Study of Heterothallism in Puccinia coronata. *J. Agr. Res.*, 45: 513.

————. 1934. A Cytological Study of Heterothallism in Flax Rust. *J. Agr. Res.*, 49: 765.

————. 1934a. A Cytological Study of Heterothallism in Puccinia sorghi. *J. Agr. Res.*, 49: 1047.

ANDRUS, C. F. 1931. The Mechanism of Sex in Uromyces appendiculatus and U. vignae. *J. Agr. Res.*, 42: 559.

COTTER, R. U. 1932. A New Form of Oat Stem Rust from a Barberry Area. *Phytopath.*, 22: 788.

COTTER, R. U. and LEVINE, M. N. 1938. Experiments in Crossing Varieties of Puccinia graminis. (Abst.) *Phytopath.*, 28: 6.

CRAIGIE, J. H. 1927. Experiments on Sex in Rust Fungi. *Nature,* 120: 116.

————. 1927a. Discovery of the Function of the Pycnidia of the Rust Fungi. *Nature,* 120: 765.

————. 1928. On the Occurrence of Pycnia and Aecia in Certain Rust Fungi. *Phytopath.,* 18: 1005.

CUMMINS, G. B. 1931. Heterothallism in Corn Rust and Effect of Filtering the Pycnial Exudate. *Phytopath.,* 21: 751.

D'OLIVEIRA, B. 1938. New Hosts for the Aecidial Stage of Uromyces graminis (Niessl) Diet. *Bol. Soc. Broteriana, Ser. 2,* 13: 81.

ERIKSSON, J. 1894. Ueber die Specialisirung des Parasitismus bei den Getreiderostpilzen. *Ber. Deut. Bot. Gesell.,* 12: 292.

JOHNSON, T., NEWTON, MARGARET and BROWN, A. M. 1932. Hybridization of Puccinia graminis tritici with Puccinia graminis secalis and Puccinia graminis agrostidis. *Sci. Agr.,* 13: 141.

————. 1934. Further Studies of Inheritance of Spore Colour and Pathogenicity in Crosses Between Physiologic Forms of Puccinia graminis tritici. *Sci. Agr.,* 14: 360.

JOHNSON, T. and NEWTON, MARGARET. 1933. Hybridization between Puccinia graminis tritici and Puccinia graminis avenae. *Proc. World's Grain Exhibition and Conference, Regina, Canada,* 2: 219.

————. 1938. The Origin of Abnormal Rust Characteristics through the Inbreeding of Physiologic Races of Puccinia graminis tritici. *Canad. J. Res., Sec. C,* 16: 38.

KAWAMURA, E. 1934. On the Function of the Spermatia of Gymnosporangium Haraeanum Syd. *Agr. and Hort.,* 9: 1299.

LAMB, I. M. 1935. The Initiation of the Dikaryophase in Puccinia phragmitis (Schum.) Körn. *Ann. Bot.,* 49: 403.

LEVINE, M. N. and COTTER, R. U. 1931. A Synthetic Production of Puccinia graminis hordei F. and J. (Abst.) *Phytopath.,* 21: 107.

LEVINE, M. N., COTTER, R. U. and STAKMAN, E. C. 1934. The Production of an Apparently New Variety of Puccinia graminis by Hybridization on Barberry. (Abst.) *Phytopath.,* 24: 13.

MAINS, E. B. 1934. Host Specialization of Puccinia sorghi. *Phytopath.,* 24: 405.

MILLAR, P. R. 1932. Pathogenicity of Three Red-Cedar Rusts That Occur on Apple. *Phytopath.,* 22: 723.

NEWTON, MARGARET, JOHNSON, T. and BROWN, A. M. 1930. A Preliminary Study on the Hybridization of Physiologic Forms of Puccinia graminis tritici. *Sci. Agr.*, 10: 721.

————. 1930a. A Study of the Inheritance of Spore Colour and Pathogenicity in Crosses Between Physiologic Forms of Puccinia graminis tritici. *Sci. Agr.*, 10: 775.

Pierson, R. K. 1933. Fusion of Pycniospores with Filamentous Hyphae in the Pycnium of the White Pine Blister Rust. *Nature,* 131: 728.

Stakman, E. C. and Piemeisel, F. J. 1917. Biologic Forms of Puccinia graminis on Cereals and Grasses. *J. Agr. Res.,* 10: 429.

Stakman, E. C., Piemeisel, F. J. and Levine, M. N. 1918. Plasticity of Biologic Forms of Puccinia graminis. *J. Agr. Res.,* 15: 221.

Stakman, E. C., Levine, M. N. and Leach, J. G. 1919. New Biologic Forms of Puccinia graminis. *J. Agr. Res.,* 16: 103.

Stakman, E. C. and Levine, M. N. 1922. The determination of Biologic Forms of Puccinia graminis on Triticum spp. *Minn. Agr. Exp. Sta. Tech. Bull.,* 8.

Stakman, E. C., Levine, M. N. and Cotter, R. U. 1930. Origin of Physiologic Forms of Puccinia graminis through Hybridization and Mutation. *Sci. Agr.,* 10: 707.

Stakman, E. C., Levine, M. N., Cotter, R. U. and Hines, L. 1934. Relation of Barberry to the Origin and Persistence of Physiologic Forms of Puccinia graminis. *J. Agr. Res.,* 48: 953.

Waterhouse, W. L. 1929. A Preliminary Account of the Origin of Two New Australian Physiologic Forms of Puccinia graminis tritici. *Proc. Linn. Soc. New South Wales,* 54: 96.

————. 1932. On the Production in Australia of Two New Physiologic Forms of Leaf Rust of Wheat, Puccinia triticina Erikss. *Proc. Linn. Soc. New South Wales,* 57: 92.

THE ORIGIN OF PHYSIOLOGIC RACES IN THE SMUT FUNGI BY HYBRIDIZATION

By H. A. RODENHISER

DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

In the past few years, rather extensive studies have been made of the smut fungi, particularly with reference to the origin of physiologic races. As these studies have progressed, the information has become not only of academic interest but of practical importance to geneticists, plant pathologists, and plant breeders who are interested in the development of smut-resistant lines of cereals. Intraspecific, interspecific, and even intergeneric hybrids have been made. As a result, new entities have developed that differ both phenotypically and genotypically from the parental lines.

Facts concerning the origin of physiologic races of the smut fungi through hybridization may perhaps be better understood if first a brief summary is given regarding the sexuality of these organisms, particularly those that commonly produce sporidia. The sexual process in these smut fungi is reduced to very simple terms. The sporidia, which are morphologically similar, are gametes and each contains a haploid nucleus. Whenever two of opposite sex are close together, fusion may take place. The nuclei migrate from their respective sporidia into an infection hypha, which develops from the fused sporidia, and remain associated throughout the development of the mycelium in the host plant. At the time of chlamydospores formation, the nuclei of opposite sex fuse, thereby forming the true diploid spore. It should be kept clearly in mind that the haploid sporidia or hyphae do not normally infect the host plant, and that it is only after what is essentially a process of hybridization that infection may take place. A sporidium of one sex may fuse with one of opposite sex (1) of another physiologic race in the same species, (2) of another species in the same genus, or even (3) of a species in another genus. In the cases where chlamydospores are produced, obviously there are combined in them factors governing a number of different characters. The results of studies of the progeny of the chlamydospores thus formed leave no doubt that new races of the smut fungi originate in this manner. Segregates are often obtained that differ widely from the parent lines in factors governing cultural characters, tendency to mutate, morphology of chlamydospores as well as in factors governing pathogenicity.

The inheritance of factors governing cultural characters from intraspecific crosses in *Ustilago zeae* (Beckm.) Ung. has been studied extensively by Stakman, Christensen, and their coworkers (1929). Factors for cultural characteristics of the progeny from a single chlamydospore were found to segregate independently of sex and of one another and, as reported by Christensen (1931), the segregation of the factors for either sex or cultural characters may be on a 4:0, 3:1, 2:2, 1:2:1, or 1:1:1:1 basis. His studies of F_1 lines on potato-dextrose agar indicate that there are multiple factors in *U. zeae* for rate of growth, type of growth, color, consistency, and topography; and possibly for the tendency to mutate. Christensen further found that 32 F_1 lines isolated from chlamydospores in a single smut gall resulting from crossing two monosporidial lines were all culturally different from each other. In a similar manner, Holton (1932) studied the segregation of factors governing cultural characters resulting from intraspecific crosses in *U. avenae* (Pers.) Jens. and *U. levis* (Kellerman and Swingle) Magnus. The haploid colonies differed as strikingly as did those of *U. zeae.* Furthermore, in five lines isolated successively from a single segment of the promycelium, four proved to be different in cultural characters.

More recently, the writer studied the cultural characters of a monosporidial line of *Sphacelotheca sorghi* (Link) Clinton and one of *S. cruenta* (Kühn) Potter in comparison with the cultural characters of the progeny of the interspecific cross between these two lines. Of 90 monosporidial lines from the progeny, only 2 appeared to be identical with the parental line of *S. sorghi* and 1 with the parental line of *S. cruenta.* The other lines of the cross were different from the parental lines in one or more of the following colony characters: color, topography, surface, consistency, margin, rate of growth, and tendency to mutate. Factors governing any one of these cultural characters segregated independently of the others and of those determining sex.

Although the subject of the origin of physiologic races by mutation will be discussed in the following paper by Dr. J. J. Christensen, it is important to point out here that during a period of three years no apparent mutation has taken place in the two haploid lines used as parents in the interspecific cross referred to above. During this time they have been subcultured at intervals of two months, and at no time have wedge-shaped sectors or patch mutants appeared. There was no visual evidence of mutation in 5 of 90 monosporidial lines. However, in each of 81 of the lines, from 2 to 4 wedge-shaped sectors or patch mutants developed. The remaining 4 lines were particularly unstable, and it was not uncommon to find in any one of the 4 colonies at least 10 distinct sectors or patches. Wide variations in color and in type and rate of growth occurred, even to the extent of a black, mycelioid, rapidly growing mutant from an almost cream colored, sporidial, relatively slow-growing line.

Hybrids may differ also from parent lines in factors other than those governing cultural characters. This was true in the progeny resulting from the interspecific cross of *Sphacelotheca sorghi* and *S. cruenta.* In the segregating progeny of a first generation backcross to the recessive parent, *S. sorghi,* phenotypes were recovered resembling, macroscopically, *S. sorghi, S. cruenta,* and a third type which was distinctly intermediate, having some of the characteristics common to both species. Some of the progeny had gray-colored peridia surrounding the sori, some had grayish brown, and still others reddish brown peridia. Other segregates were strikingly different in the degree to which they stunted the host plant. Allison (1937) found that *Ustilago hordei* (Pers.) Kellerman and Swingle, the pathogen causing the covered smut of barley, hybridized readily with the loose smut organism, *U. medians* Bieden, or *U. nigra* Tapke. Chlamydospores of the covered smut fungus are smooth and those of the loose smut fungus are echinulate. In the F_1 generation, the chlamydospores were found to be echinulate and the head type intermediate. Combinations of head type and chlamydospore wall markings differing from either parent type were obtained in the F_2 generation.

New entities have been produced also as a result of hybridization between smut fungi of different genera. Tyler and Shumway (1935) crossed *Sphacelotheca sorghi* with *Sorosporium reilianum* (Kühn) McAlpine, the pathogens causing the covered kernel and the head smuts of sorghum, respectively. Both the sori and chlamydospores produced appeared to be intermediate between the parent lines. Likewise, Vaheeduddin (1936) obtained a fertile intergeneric hybrid by crossing monosporidial lines of *Sphacelotheca cruenta* and *Sorosporium reilianum.* Here again phenotypes were obtained having some characters common to both parents. Echinulate hybrid chlamydospores were intermediate in size between both parents and produced promycelia and sporidia significantly larger than those of the parental lines.

In the process of segregation and recombination of factors affecting the cultural and morphologic characteristics, there is equal opportunity for a recombination of factors affecting pathogenicity. As a result, one might expect some of the progenies to differ in virulence from the parental lines used in the crosses. This has proved true in a number of intra- and interspecific hybrids. Christensen (Stakman, Christensen, Eide, and Peturson, 1929) found that dikaryophytes of *Ustilago zeae* resulting from pairing F_1 lines had various degrees of pathogenicity: some were very virulent, some moderately virulent, and others appeared to be relatively innocuous. In experiments with the sorghum smut organisms, at least four pathogenically distinct races have been produced from intra- and interspecific crosses of *Sphacelotheca sorghi* and *S. cruenta.* Intraspecific crosses were significantly more virulent on Reed kafir than either parent, while interspecific hybrids in these tests were less virulent than either parent. Some of the most significant results have been reported by Holton (1936a) in comparing the pathogenicity of pure lines, hybrids, and hybrid segregates of the oat smut fungi, *U. levis, U. avenae,* and the so-called buff smut organism which resulted from a mutation in *U. levis.* His data (Holton 1936) presented in Table I indicate clearly that a new pathogenic strain of *U. levis* that attacks the varieties Gothland and Monarch was produced by crossing a Gothland strain of *U. avenae* with a Monarch strain of *U. levis.* Also it is evident that a new pathogenic strain of the buff smut fungus that attacks both Gothland and Monarch was produced by crossing the Gothland strain of *U. avenae* with the Monarch buff smut strain which would not infect Gothland. Holton (1938) reported also on the pathogenicity of an interspecific hybrid resulting from a cross between the bunt smut organisms, *Tilletia tritici* (Bjerk.) Wint. (race T–9) and *T. levis* Kühn (race L–8). The variety Oro is resistant to T–9 and susceptible to L–8. Hohenheimer is susceptible to T–9 and resistant to L–8. The hybrid obtained as a result of the cross, although less virulent than either parent, attacked both varieties sufficiently to distinguish it from either of the parent races. It is significant that the hybrid selection which produced 46 per cent bunt on Oro resembled *T. tritici* in morphology, whereas this variety has proven highly resistant to all of the races of this species described by Rodenhiser and Holton (1937).

In the experiments that have been cited, hybrid lines were produced under carefully controlled laboratory conditions. The question arises, however, as to whether there is opportunity for intra- and interspecific hybridization to take place under natural conditions in the field. Undoubtedly there is opportunity, particularly where races and species having the same life histories are found in the same field. This is frequently the case as regards races and species of *Tilletia.* In collections of approximately 25 bunt-infected heads from a single field, it is not uncommon to find both *T. levis* and *T. tritici* present and both having entirely different factors for pathogenicity. Furthermore, in these collections, race and species mixtures are occasionally found in the same spike and even the same bunt ball. In some of our recent tests, inoculum obtained from a single spike contained a race of *T. tritici* and a race of *T. levis* which differed in pathogenicity on Marquis wheat. In addition, there is preliminary evidence that

TABLE I

Percentages of Smutted Panicles Produced on Oat Varieties by Pure Lines, Hybrids, and Hybrid Segregates of Ustilago levis, U. avenae, and the Buff Smut Fungus*

Variety of oats	Percentage of panicles smutted by						
	U. levis	*U. avenae*	Buff smut	*U. levis* × *U. avenae*		*U. avenae* × buff smut	
				F_2	F_2 *U. levis* segregate F_3	F_2	F_2 buff segregate F_3
Gothland	0.0	100.0	0.0	22.0	92.0	3.0	100.0
Monarch	99.0	.0	55.0	21.0	45.0	6.0	22.0

* The low percentages of smut produced in the F_2 of the *U. levis* × *U. avenae* and *U. avenae* × buff smut hybrids is no doubt explainable on the basis of a low degree of viability of F_2 sporidia and the production of individuals, through segregation and recombination of factors for pathogenicity, that were not pathogenic on these two varieties.

chlamydospores obtained from a third smut ball of the same head are of a race of *T. levis* pathogenic to the variety Hohenheimer and not previously identified in smut collections in this country. There is likewise opportunity for hybridization between *Ustilago avenae* and *U. levis.* These two species of oat smut, which have the same life history, are often found in the same fields, and it seems significant that among them it is not uncommon to find intermediate types having some of the characteristics of both the loose and covered type smuts. The loose and covered kernel smuts of sorghum may also be found in the same fields, and there is some evidence, according to Tisdale, Melchers, and Clemmer (1927), that their collection of the milo race of sorghum smut originated as a result of hybridization between *S. sorghi* and *S. cruenta.* Although classified as a race of *S. sorghi,* the original collection had macroscopic and microscopic characteristics common to both species. The general morphology of the intermediate types which have been produced in the laboratory by interspecific hybridization are in every way similar to the one given by Tisdale *et al.* (1927) for the appearance of their milo race and lends support to their explanation regarding the origin of the race.

In this discussion an attempt has been made to point out that, in laboratory experiments, fusions readily occur between haploid lines of different races, species, and genera, thus initiating the formation of diploid hyphae containing hereditary factors of two parental lines. As a result of recombination and segregation of these factors, new entities have developed that differ from the parent lines in cultural characteristics, morphology, and pathogenicity. It has been further pointed out that there is ample opportunity for hybridization to take place under field conditions. Mixtures of races and species of the organisms having the same life histories are commonly found in the same commercial grain fields, and it is not unusual to find types having characteristics common to two different species. This is, of course, circumstantial evidence that hybridization has occurred. However, the ease with which hybrids may be made under controlled conditions and the similarities of these progenies with those intermediate types found in the field are certainly strong evidence that physiologic races of the smut fungi occur in nature as a result of hybridization.

References Cited

Allison, C. C. 1937. Studies on the Genetics of Smuts of Barley and Oats in Relation to Pathogenicity. *Minn. Agr. Exp. Sta. Bull.,* 119.

Becker, T. 1936. Untersuchungen über Sexualität bei Tilletia tritici (Bjerk.) Wint. im Rahmen der Immunitätszüchtung. *Phytopath. Zeitschr.,* 9: 187.

Christensen, J. J. 1931. Studies on the Genetics of Ustilago zeae. *Phytopath. Zeitschr.,* 4: 129.

Dickinson, S. 1927. Experiments on the Physiology and Genetics of the Smut Fungi.—Seedling Infection. *Proc. Roy. Soc. London, B,* 102: 174.

Flor, H. H. 1932. Heterothallism and Hybridization in Tilletia tritici and T. levis. *J. Agr. Res.,* 44: 49.

Hanna, W. F. and Popp, W. 1930. Relationship of the Oat Smuts. *Nature,* 126: 843.

Holton, C. S. 1932. Studies in the Genetics and the Cytology of Ustilago avenae and Ustilago levis. *Minn. Agr. Exp. Sta. Tech. Bull.,* 87.

————. 1936. Origin and Production of Morphologic and Pathogenic Strains of the Oat Smut Fungi by Mutation and Hybridization. *J. Agr. Res.,* 52: 311.

————. 1936a. Inheritance of Chlamydospore Characteristics in Oat Smut Fungi. *J. Agr. Res.,* 52: 535.

————. 1938. A New Pathogenically Distinct Race Derived from a Cross between Tilletia tritici and T. levis. *Phytopath.,* 28: 371.

Kniep, H. 1926. Über Artkreuzungen bei Brandpilzen. *Zeitschr. f. Pilzkunde,* 5: 217.

Rodenhiser, H. A. 1932. Heterothallism and Hybridization in Sphacelotheca sorghi and S. cruenta. *J. Agr. Res.,* 45: 287.

————. 1935. Studies on the Possible Origin of Physiologic Forms of Sphacelotheca sorghi and S. cruenta. *J. Agr. Res.,* 49: 1069.

Rodenhiser, H. A. and Holton, C. S. 1937. Physiologic Races of Tilletia tritici and T. levis. *J. Agr. Res.,* 55: 483.

Stakman, E. C., Christensen, J. J., Eide, C. J. and Peturson, B. 1929. Mutation and Hybridization in Ustilago zeae. *Minn. Agr. Exp. Sta. Tech. Bull.,* 65.

Tisdale, W. H., Melchers, L. E. and Clemmer, H. J. 1927. Strains of Kernel Smuts of Sorghum, Sphacelotheca sorghi and S. cruenta. *J. Agr. Res.,* 34: 825.

Tyler, L. J. and Shumway, C. P. 1935. Hybridization between Sphacelotheca sorghi and Sorosporium reilianum. *Phytopath.,* 25: 375.

Vaheeduddin, S. 1936. Hybridization between Sphacelotheca cruenta and Sorosporium reilianum. (Abst.) *Phytopath.,* 26: 111.

THE ORIGIN OF PARASITIC RACES OF PHYTOPATHOGENIC FUNGI THROUGH MUTATION[1]

By J. J. CHRISTENSEN[2]

DIVISION OF PLANT PATHOLOGY AND BOTANY, UNIVERSITY OF MINNESOTA, ST. PAUL, MINN.

Craigie and Rodenhiser in papers preceding this one have presented evidence that new races of pathogenic fungi may arise as the result of hybridization between biotypes. New races also may arise in fungi that reproduce asexually and in which hybridization does not occur or, at least, does not play an important rôle. Moreover, they arise frequently in haploid lines of heterothallic fungi. Obviously, some explanation other than hybridization and adaptation is necessary to account for these variations. Such changes probably can best be explained on the basis of mutations or saltations.

Mutation may be defined as a genotypic change which is not the result of hybridization and segregation, and which is heritable. This definition assumes that the isolate is a clon and preferably derived from a single nucleus. It is significant that derivation of a line from a single spore is no assurance of genetic purity, as diploid, dicaryotic, and heterocaryotic spores may give rise to two or more biotypes on germination.

It has been generally recognized that mutations are common in both haploid and diploid lines of fungi, including the smuts (Christensen 1932; Rodenhiser 1934; Stakman *et al.* 1929). It seems logical to assume that mutation may also occur in races of fungi derived from multinucleate spores. Objections have been raised to the designation of variants arising in Fungi Imperfecti as mutants because, in many species, the cells and spores are multinucleate, and consequently variants might arise by the separation of genetically unlike nuclei (Brierley 1929; Hansen 1938). There is evidence that sometimes this actually happens (Dickinson 1932; Hansen and Smith 1932; Hansen 1938). But heterocaryosis apparently does not explain some of the variation which it originally was supposed to explain. Even though spores may be multinucleate, it does not follow that the nuclei contain different genetic factors. In fact, it has been shown that the nuclei in a multinucleate spore in some fungi are derived from a single nucleus and are therefore genetically alike unless reduction division occurred or unless there had been some chromosomal abnormality, resulting in mutation (Christensen and Davies 1937; Dickinson 1932). In *Helminthosporium sativum* P.K.B., for example, one of the most mutable of the Fungi Imperfecti with which the writer is familiar, all of the nuclei in conidia produced on the same conidiophore are derived from a single nucleus (Christensen and Davies 1937). Extensive experiments were made to ascertain whether variation in this fungus might be due to chance assortment of nuclei, but the results were negative. All indications were that the spores themselves were not in reality heterocaryotic and therefore their variation could hardly result from a chance assortment of nuclei.

Variability

It is important to distinguish clearly between phenotypic modification or variability and true genetic changes. Fluctuations in growth, color changes, amount of fructification, and changes in other cultural characters due to temperature, nutrients, chemicals, hydrogen-ion concentra-

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[1] Paper No. 1726 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] In a short review it is impossible to discuss all of the results pertinent to this problem, hence, the writer, on account of his greater familiarity with the work done at University Farm, has relied chiefly on these results. The writer is indebted to Professor E. C. Stakman for help and criticism in preparation of this manuscript.

tions, and the presence of toxic materials are common examples of variability which disappear when the stimulus is removed. There is no evidence that fungi like *Ustilago zeae* (Beckm.) Ung. and *Helminthosporium sativum* lose or acquire new characters gradually as a result of growth on different media or under varying conditions in the laboratory. Changes do occur in cultural characteristics and in pathogenicity, but they arise suddenly and are heritable. In certain fungi it is not uncommon to find a monosporous culture which has bred true for several cultural generations, both from spores and mycelium, giving rise to one or more variants differing strikingly from the rest of the culture.

FREQUENCY OF MUTATION

Mutation has been reported in all classes of fungi (Christensen 1926; Das Gupta 1936; Stakman 1928; Stevens 1922). There are marked differences in mutability, not only among different groups, but also among species belonging to the same genus. For instance, mutation is very common in *Helminthosporium sativum, Phlyctaena linicola* Speg., and *Sphacelotheca sorghi* (Lk.) Cl., but it apparently is relatively infrequent in *Rhizoctonia solani* Kühn and *Puccinia graminis* Pers. (Christensen 1926; Newton and Johnson 1927; Rodenhiser 1930; Stakman 1928; Stakman *et al.* 1930; Vallega 1936). Even biotypes within a species may differ greatly in their tendency to mutate (Christensen 1926, 1932; Stakman *et al.* 1929; Stakman 1936).

During the past ten years, the writer has observed in the laboratories at University Farm, St. Paul, Minnesota, thousands of variants involving many species. Many of these have been isolated and cultured for many clonal generations, while some have been hybridized and their progeny studied. From two unisexual lines of *Ustilago zeae,* Stakman[3] and his coworkers have isolated hundreds of new haploid biotypes. The production of so many mutants may seem almost incredible, yet the frequency of mutation probably is not greater than that in some higher plants. This is apparent when one remembers that a single colony of smut 25–30 mm in diameter may consist of billions of individuals (sporidia). Stakman (1936) has proven that the tendency to produce mutants in *U. zeae* is determined by genetic factors.

HOW MUTANTS ARISE

Mutants may arise either in artificial culture or on living hosts. They most frequently arise as wedge-shaped or fan-shaped sectors in colonies growing on nutrient media, but sectors of various other shapes are not unusual. The relative proportion of a colony occupied by a mutant or mutants and the original type are quite variable. Sectors may arise at one or more points, sometimes in masses over a considerable area of a colony. The margin of a colony of *Helminthosporium sativum* may be occupied almost entirely by sectors of different colors, sizes, and shapes. The total number of sectors produced in a colony derived from a single spore may vary considerably. More than 40 sectors have been counted in a single colony of *H. sativum* growing in a petri dish.

Mutants also arise in tufts or "patches" of various types on the surface of colonies. These "patch" mutants are often difficult to detect and hence probably are much more common than is generally supposed. In some fungi, mutants may actually grow over the greater part of the parental colony. Furthermore, they can not always be detected, as some arise that are indistinguishable from the parent culturally and morphologically, although they may differ in physiology and pathogenicity. Inoculations made from either type of culture, which in reality has become a mixture of biotypes, may lead to erroneous conclusions regarding loss or increase in sporulating ability, decrease or increase in virulence, etc.

KINDS OF MUTANT CHARACTERS

The range of mutant characters is very wide in some fungi. The mutants may differ from their parents in cultural characters, in physiologic characters, in pathogenicity, and even in morphology. Furthermore, they may differ from each other decidedly in their tendency to mutate. Obviously, those mutants that are most distinct from the parents attract attention most quickly and are likely to be most extensively studied. While some mutants may clearly have the general characters of the species, they are distinct and easily recognized biotypes. That is, there may be within a species a very large number of biotypes that differ from each other in one or more characters but which have not transcended specific limits. Other mutants, however, are so different from their parents in one or more characters as to make it justifiable to consider them as different species on all grounds except strictly utilitarian ones (Brown 1926; Christensen 1926; Mitter 1929). There is at least one case on record in which mutants of one species would have to be put into a different genus under the accepted system of classification. From a monosporous culture of *Pestalozzia funerea* Desm., C. Christensen (1932) isolated mutants which had only a single, short, stubby appendage instead of the three long appendages of the parental type. The mutant therefore on the basis of morphology belongs in the genus *Monochaetia.* Mutants that differ decidedly from their parents are easy to detect, but in many fungi numerous mutants may arise that differ in only very minor characters from their parents. In fact, in *Ustilago zeae* and various other fungi, mutants may differ from their parents only in slight shades of color, in almost imperceptible differences in the topography of colonies, and other similar characters. Yet it has been demonstrated that these very minor characters are definitely determined by genetic factors.[4] Because of their number and difficulty of detection, it is entirely possible that these minor mutations may be even more important than the more spectacular ones. It is entirely possible that there are many mutations in physiologic characters only. These of course may not necessarily give visual evidence of their existence so that conclusions might be drawn regarding changes in virulence or other physiologic characters resulting from growth on certain media or in certain hosts, particularly if the substratum has a selective effect on the different biotypes resulting from mutation in a given line or culture.

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[3] Unpublished data.

That mutants may differ from their parents in cultural characters has been demonstrated repeatedly. They may differ from their parents in tolerance to dyes and toxic substances, in the production of acids and enzymes, and in temperature relations (Christensen and Davies 1937; Das Gupta 1936; Dimock 1936; Stakman *et al.* 1929). Mutation in physiologic characters may be accompanied also by mutation in cultural characters. Mutants often differ from their parents and from each other in rate of growth, type of growth, color of colonies, surface of colonies, topography of colonies, and in amount of sporulation (Christensen 1926; Leonian 1932; Mitra 1931; Mitter 1929; Stevens 1922; Vallega 1936). Changes in spore size and character of mycelium as a result of mutation also have been reported for several fungi (Brown 1926; Das Gupta 1936; Dickson 1933; Mitter 1929; Wiltshire 1929).

In a few cases comparative studies have been made of the virulence of mutants and their parents (Bonde 1929; Christensen 1929; Mitter 1929; Rodenhiser 1930; Stakman *et al.* 1929, 1930). A mutant in *Helminthosporium sativum* usually had the same pathogenicity as its parent. When a change in pathogenicity did occur, the mutant was usually less virulent than the parent, while some were non-pathogenic and others were distinctly more virulent than the parent (unpublished data and Christensen 1929). Definite genetic changes in pathogenicity also have been demonstrated in mutants from haploid lines of *Ustilago zeae* (Stakman *et al.* 1929, 1933). The increase in virulence of mutants arising in nutrient media is rather significant, as it may possibly explain the apparent increase in pathogenicity which sometimes results from the passage of a culture through a living host.

Mutation for sex may occur in some heterothallic fungi. Certain mutants of

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[4] E. C. Stakman's unpublished data on *Ustilago zeae.*

Ustilago zeae, for example, lost their ability to combine with certain lines of an opposite sex, but retained their ability to combine with others (Stakman *et al.* 1929). It is entirely possible, therefore, that apparent loss of pathogenicity in some heterothallic fungi may sometimes be due to loss of factors for sex rather than of factors for pathogenicity as such.

Stability of Mutants

There are various degrees of stability of mutants. Many are as unstable as their parents and continue to give rise to numerous mutants in every cultural generation. Others remain constant for a few generations and then occasionally produce a mutant; still others are quite stable and mutate rarely or not at all. Mutants of *Helminthosporium sativum* and their parental types have been grown side by side in the same petri dishes and have retained their distinctive characters. Some have been transferred repeatedly on various nutrient media for a period of years, and have remained as distinct in cultural characters as they were the day they were originally isolated (Christensen 1929). Studies on the stability of mutants of *Ustilago zeae* over a period of years have given similar results (Stakman *et al.* 1933).

All sectors, especially those that occur on margins of old colonies, are not necessarily mutants. On subculturing, such sectors may revert to the parental type and are commonly termed "false sectors" (Vasudeva 1930). It is not surprising that reversions have often been reported, for the genetic purity of variants has not always been established. Sectors are not necessarily pure for type because mutants may grow either over or under the parent, and subcultures from such sectors may consist of the mutant plus the parental line. There is considerable evidence which indicates that true reversions do occur, and there is no reason why they should not.

The stability of mutants of *Helminthosporium sativum* also has been determined on living hosts (Christensen 1929). The cultural characters of the variants which were passed through the host, with one exception, remained unchanged. Their sojourn on the living host did not change their pathogenicity. Obviously, mutation of cultural characters in *H. sativum* may occur on the living host, and there is no valid reason why mutation for virulence also should not occur there. There is need for critical experiments on the effects of passing pathogens through living hosts.

Factors Affecting Mutation

Numerous tests with many fungi indicate that frequency of mutation can be altered profoundly by such environmental factors as temperature, light, nutrients, hydrogen-ion concentration, and addition of chemicals and toxic materials to the substrate.

The concentration and the kind of nutrient used are of great importance in inducing mutation. Certain fungi seldom mutate when grown on ordinary potato-dextrose agar, but mutate readily on special media (Brown 1926; Christensen 1926; Das Gupta 1936; Stakman *et al.* 1929). Some species of *Helminthosporium* and *Fusarium* may produce many mutants on one medium, while others on that same medium do not mutate. On a second medium the tendency of the species to mutate may be the opposite. The same relationship may exist among races of fungi within a species or mutants derived from a given race (Brown 1926; Christensen 1929, 1932; Stakman *et al.* 1929).

The addition of certain salts and antiseptics to nutrient media is known to stimulate the production of variants (Dimock 1936; Galloway 1933; Sibilia 1934; Stakman *et al.* 1929). Recently it has been found that the addition of New Improved Ceresan (ethyl mercury phosphate) at the rate of 4 parts per million to potato-dextrose agar increased the frequency of mutation in certain lines of *Helminthosporium sativum* but did not stimulate mutation in about 20 other fungi.

Several workers have been able to induce variation by exposing fungus cultures and spores to high temperatures; thus, the stability of *Helminthosporium sativum* and

Ustilago zeae was greatly modified by temperature, cultures being unstable at high temperatures and stable at low temperatures (Christensen 1929; Mitra 1931; Stakman *et al.* 1929). *Eurotium herbariorum* (Wigg.) Lk. well exemplifies a fungus from which numerous variants have been obtained as a result of exposing its spores to high temperatures (Barnes 1928, 1936).

Ultraviolet rays, X-rays, and radium radiation have been used successfully for inducing mutation. Some fungi respond readily to one or more of these treatments, while others do not (Das Gupta 1936; Dickson 1932, 1933; Greaney and Machacek, 1933).

Staling products from fungi or bacteria may inhibit or induce mutation. Brown (1926) concluded that the presence of a staling product in a medium which materially retarded growth reduced the frequency of mutation. It has been known for some time that certain bacteria are decidedly antibiotic to *Helminthosporium* (Porter 1924). Recently it has been shown that *Bacillus mesentericus* (Flügge) Migula not only retards growth of *H. sativum* but stimulates the production of mutants (Christensen and Davies 1937). Thus, the addition of 1 or 2 per cent of a dead broth culture of *B. mesentericus* to potato-dextrose agar greatly increased the frequency of mutation and the type of mutant produced. On the staled medium, many isolates of *H. sativum* produced from 1 to 5 variants per colony and some developed many more, 10–15 per colony being not uncommon. These induced variants differed not only in cultural characters, but also in physiological characters, such as tolerance to dyes and to the bacterial toxins. In fact, it was possible to select lines that grew normally at a far higher concentration of a toxic bacterial product than their parents. Some, however, were much more sensitive to it. Additional proof that these induced changes were genetic, rather than mere modifications, is indicated by changes in parasitism. Some of the mutants were more virulent than their parents, others were less virulent, and still others were non-pathogenic.

The results of various tests indicate that certain fungi may not mutate under ordinary laboratory conditions but may do so freely when subjected to special stimuli. No generalization, however, can safely be made with respect to the action of these stimuli in inducing mutation. The effect of certain stimuli may be specific for different species of fungi and even for biotypes within a species. For example, products of bacterial growth may stimulate certain lines of *Helminthosporium* to mutate freely but may be without effect on others.

Conclusions

It seems reasonable to conclude that variants, which are the result of genetic changes and are not mere modifications—temporary phenotypes or *Dauermodifikationen*—induced by environmental factors, arise in culture and in the host. It is likely that some variants recorded in the literature are the result of segregation from a sexual spore or dissociation of biotypes from a heterocaryotic race. Because of the minuteness of nuclei, the chromosomal behavior is difficult to study. As a consequence, the exact nature of the change or changes remains obscure, but there is considerable circumstantial evidence that indicates some nuclear change: the production of many different types from a single monosporous line, especially from one derived from a haploid uninucleate cell; the stability of these variants on different nutrients over a period of years and on living hosts; and their breeding behavior. The best proof of a genetic change is hybridization and a study of the progeny. Such studies are not possible with imperfect fungi, but they are possible with mutants arising in heterothallic lines. Hybridization of variants in *Ustilago zeae* has proved that new characters are inherited through the sexual stage (Christensen 1932; Stakman *et al.* 1929).

Heterothallic fungi, especially the smuts, are desirable material for the study of mutation because they can be grown under extreme conditions and under very exact environments, and because, under these conditions, even minute changes can be detected readily. Also, many asexual and sexual generations of large populations can be grown in a relatively short period of time and in a small space. Moreover, mutants can be readily crossed and the progeny studied in the laboratory, greenhouse, or field. Their use as laboratory material for the study of mutation in fungi may add much to our knowledge of fundamental genetics and to the understanding of evolution.

References Cited

Barnes, B. 1928. Variations in Eurotium herbariorum (Wigg.) Link. Induced by the Action of High Temperatures. *Ann. Bot.*, 42: 783.

———. 1936. Presidential Address. Induced Variation. *Trans. Brit. Myc. Soc.*, 20: 17.

Bonde, R. 1929. Physiological Strains of Alternaria solani. *Phytopath.*, 19: 533.

Brierley, W. B. 1929. Variation in Fungi and Bacteria. *Proc. Intern. Congr. Plant Sci., Ithaca*, 2: 1629.

Brown, W. 1926. Studies in the Genus Fusarium. IV. On the Occurrence of Saltations. *Ann. Bot.*, 40: 223.

Christensen, C. 1932. Cultural Races and the Production of Variants in Pestalozzia funerea. *Bull. Torrey Bot. Club*, 59: 525.

Christensen, J. J. 1926. Physiologic Specialization and Parasitism of Helminthosporium sativum. *Minn. Agr. Exp. Sta. Tech. Bull.*, 37.

———. 1929. The Influence of Temperature on the Frequency of Mutation in Helminthosporium sativum. *Phytopath.*, 19: 155.

———. 1932. Studies on the Genetics of Ustilago zeae. *Phytopath. Zeitschr.*, 4: 129.

Christensen, J. J. and Davies, F. R. 1937. Nature of Variation in Helminthosporium sativum. *Mycologia*, 29: 85.

Das Gupta, S. N. 1936. Saltation in Fungi. Lucknow University Studies V, 1934–35. Newul Kishore Press, Lucknow.

Dickinson, S. 1932. The Nature of Saltation in Fusarium and Helminthosporium. *Minn. Agr. Exp. Sta. Tech. Bull.*, 88.

Dickson, H. 1932. The Effects of X-rays, Ultraviolet Light, and Heat in Producing Saltants in Chaetomium Cochliodes and Other Fungi. *Ann. Bot.*, 46: 389.

———. 1933. Saltation Induced by X-rays in Seven Species of Chaetomium. *Ann. Bot.*, 47: 735.

Dimock, A. W. 1936. Variation in a Species of Fusarium Induced by High Concentration of Zinc Salts. *Zentralbl. f. Bakt.*, II, 95: 341.

Galloway, L. D. 1933. The Stimulation by Dilute Antiseptics of "Sectoring" in Mould Colonies. *Trans. Brit. Myc. Soc.*, 18: 161.

Greaney, F. J. and Machacek, J. E. 1933. Production of a White Fertile Saltant of Helminthosporium sativum by Means of Ultraviolet Radiation. *Phytopath.*, 23: 379.

Hansen, H. N. and Smith, R. E. 1932. The Mechanism of Variation in Imperfect Fungi: Botrytis cinerea. *Phytopath.*, 22: 953.

Hansen, H. N. 1938. The Dual Phenomenon in Imperfect Fungi. *Mycologia*, 30: 442.

Leonian, L. H. 1932. The Pathogenicity and the Variability of Fusarium moniliforme from Corn. *West Virginia Agr. Exp. Sta. Bull.*, 248.

Mitra, M. 1931. Saltation in the Genus Helminthosporium. *Trans. Brit. Myc. Soc.*, 16: 115.

Mitter, J. H. 1929. Studies in the Genus Fusarium. VII. Saltation in Section Discolor. *Ann. Bot.*, 43: 379.

Newton, Margaret and Johnson, T. 1927. Color Mutations in Puccinia graminis tritici (Pers.) Erikss. and Henn. *Phytopath.*, 17: 711.

Porter, C. L. 1924. Concerning the Characters of Certain Fungi Exhibited by Their Growth in the Presence of Other Fungi. *Amer. J. Bot.*, 11: 168.

Rodenhiser, H. A. 1930. Physiologic Specialization and Mutation in Phlyctaena linicola Speg. *Phytopath.*, 20: 931.

———. 1934. Studies on the Possible Origin of Physiologic Forms of Sphacelotheca sorghi and S. cruenta. *J. Agr. Res.*, 49: 1069.

Sibilia, C. 1934. "Saltazioni" in Heterosporium gracile. *Boll. Staz. Pat. Veg.*, N. S., 14: 447.

Stakman, E. C. 1928. Racial Specialization in Plant Disease Fungi. Mayo Foundation Lectures, 1926–27. W. B. Saunders Co., Philadelphia.

———. 1936. The Problem of Specialization and Variation in Phytopathogenic Fungi. *Genetica*, 18: 372.

———. 1937. Variation in Ustilago zeae. (Abst.) *Science*, 85: 58.

Stakman, E. C., Christensen, J. J., Eide, C. J. and Peturson, B. 1929. Mutation and Hybridization in Ustilago zeae. *Minn. Agr. Exp. Sta. Tech. Bull.*, 65.

Stakman, E. C., Levine, M. N. and Cotter, R. U. 1930. Origin of Physiologic Forms of Puccinia graminis through Hybridization and Mutation. *Sci. Agr.*, 10: 707.

Stakman, E. C., Tyler, L. J. and Hafstad, G. E. 1933. The Constancy of Cultural Characters and Pathogenicity in Variant Lines of Ustilago zeae. *Bull. Torrey Bot. Club*, 60: 565.

Stevens, F. L. 1922. The Helminthosporium Footrot of Wheat, with Observations on the Morphology of Helminthosporium and on the Occurrence of Saltation in the Genus. *Bull. Illinois State Nat. Hist. Surv.*, 14: 77.

Vallega, J. 1936. El Helminthosporium sativum P. K. y B. como parásito del trigo, en la República Argentina. *Agronomía*, 29(152): 43.

Vasudeva, R. N. S. 1930. On the Occurrence of "False Sectors" in Cultures of Fusarium fructigenum Fr. *Trans. Brit. Myc. Soc.*, 15: 96.

Wiltshire, S. P. 1929. A Stemphylium Saltant of an Alternaria. *Ann. Bot.*, 43: 653.

SUMMARY

By E. C. STAKMAN

VIRUSES and species of micro-organisms pathogenic to animals and plants commonly comprise several or many types, strains, biotypes, or races that may differ in pathogenicity and in morphologic or physiologic characters.

Specific virus diseases of animals, such as foot and mouth disease, South African horse sickness, and equine encephalomyelitis, are caused by immunologically distinct types or strains; and there also are closely related strains of plant viruses. Likewise, it has long been known that there are many strains of bacteria that cause animal diseases; and races of micro-organisms that cause plant diseases have been recognized for so long that their existence is taken for granted and scarcely requires further discussion. Within species of protozoa parasitic to animals there also are strains that may differ in structural and physiologic characters. Many species of fungi that cause plant diseases comprise a great many biotypes or groups of biotypes that may differ in cultural characters on artificial media, in morphology, in physiologic characters, and in pathogenicity.

The data given in the papers of this series clearly show that many or most species of pathogenic organisms, whether belonging to the animal or plant kingdom, are a composite of several or many "types," "biotypes," or "strains." If the term biotype is defined as "all individuals with identical genetic constitution," its use should perhaps be limited to theoretical considerations and to those cases in which the facts are sufficiently well known to justify its use in connection with a "line," "type," "strain," or "race." As the term race connotes similarity of origin and essential similarity of appearance and behavior, perhaps it is one of the most suitable of those commonly used to designate the recognizable components of a variety or species. However, usage varies in the different fields, and, regardless of the terms used, the fact stands out clearly that investigators of virtually all kinds of pathogenic organisms realize the importance of races or strains within a species. Obviously, all races within the species must have one or more distinctive characters in common; otherwise they would not belong properly in the same species. On the other hand, they may differ so profoundly in characters affecting pathogenicity, epidemiology, and therapy that information regarding their identity, their behavior, and their origin is of paramount importance in understanding and controlling diseases.

It is of course important to know the races within a species. In animal and human pathology the differences in virulence and antigenic properties are of great importance in understanding epizootics and epidemics and in the preparation of immune sera. For example, recovery from a specific virus infection in animals often is accompanied by a certain degree of immunity against reinfection, but if there are several strains protection is against only one strain and slight or partial against other strains of the same group. In human diseases, too, methods of immunization and diagnosis must take into consideration strain or race differences. In the preparation of vaccines against diseases caused by the typhoid and paratyphoid bacteria and other members of this group it is necessary to take into consideration the complex antigenic composition due to the occurrence of strains. Similarly, in preparing antisera against pneumococci and meningococci the existence of strains and the limits within which they vary constitute an important problem.

The existence of numerous races of plant pathogens complicates the problem of developing resistant varieties of crop plants. A variety may be highly resistant to certain races of a pathogenic fungus, for example, and completely susceptible to others. It is known that the prevalence and distribution of these races may vary with the season and with the region or locality. Therefore a given variety may be resistant in one region and susceptible in another in the same year; or it may be either susceptible or resistant in the same locality in different years, depending on the prevalence of certain races of the pathogen. This is so well known with respect to the extremely destructive stem rust of wheat and other destructive diseases that many plant breeders now create artificial epidemics in their breeding nurseries by inoculating with all available races of the pathogen. If this is not done, resistant varieties may "lose their resistance," as has often been true in the past, because the new variety is susceptible to one or several parasitic races of the pathogen against which it had not been tested in the process of its production. It is known also that the severity of rust epidemics in given regions depends on the interaction between particular races of the pathogen, particular varieties of crop plants, and the complex effects of environmental factors on both and on the interactions between them.

The existence of so many strains or races within species of pathogenic organisms is important enough in itself. It means that in controlling diseases by immunization (in plants by the production of resistant varieties) one often is confronted with many pathogens, not with one. But the situation is complicated still further because the strains themselves may vary in important characters or entirely new strains may arise. Therefore one of the most fundamental and important problems in connection with the diseases caused by pathogens, including viruses, is to learn how many strains there are, how much they can vary phenotypically and genotypically, and whether new strains come into existence through adaptation, mutation, and hybridization.

The problem of variation in its broadest sense is essentially the problem of the genetics of pathogenic micro-organisms. And the question how much a given line can vary, whether new strains or races arise as a result of adaptation to a new environment, or whether they arise fortuitously is very important. It is evident from the papers in this symposium that further attempts must be made to distinguish between phenotypic variability and genetic variation.

It is quite clear that there is conflicting evidence regarding the nature of variation in pathogens. In general, students of micro-organisms pathogenic to animals have adduced evidence for adaptation and the possible origin of temporary, semipermanent, or permanent strains in this way. Plant pathologists, on the other hand, emphasize the origin of new biotypes as a result of mutation and hybridization, with the environment exerting merely a selective effect, except as certain environmental factors may stimulate mutation. Possibly fundamental differences in the two groups of pathogens and hosts account for the differences in viewpoints. Certainly higher animals and higher plants react differently to infection, the defensive mechanisms apparently being quite different. Probably the most important difference is the ability of animals to produce specific antibodies, while plants apparently either produce them less readily or not at all. It is true that many investigators of plant diseases claim the existence of antibodies of various kinds, but the evidence does not seem unimpeachable. There is relatively meager evidence only that disease attack in plants confers immunity against subsequent attacks. The importance of such a difference in serial passages and similar procedures is obvious. It might be expected that the pathogenic viruses, bacteria, and fungi, however, would be essentially alike with respect to genetic behavior, regardless of whether they attack animals or plants, except insofar as specific antibodies in animals might affect them more than any specific substances in plants. Protozoa may become resistant to the defense mechanisms (antibodies) of their animal host. The acquisition of antibody resistance is associated with a variation in antigenic composition of the parasite.

It has been pointed out in several papers that lines of pathogens used for genetic studies should originate from single cells or single individuals of known nuclear condition to be sure that apparent changes are not due to the selective effect of environment on a mixture of strains. The importance of knowing the genetic constitution of the host, the experimental animals or host plants, also is evident. In this respect plants are more suitable for experimentation than animals, because selfing is possible and relative homozygosity is more readily attained. Furthermore, many plants can be propagated vegetatively, so that all individuals of the resulting clon are genetically alike, unless mutation occurs. Among higher animals, on the other hand, sexual reproduction is the only method of reproduction, and, unless inbreeding is practiced, the resulting populations are likely to be very heterogeneous. It is generally recognized by investigators of the genetics of plant pathogens that genotypic uniformity of the experimental host material is as essential as that of the pathogen in order to interpret results correctly. The paper by Taliaferro and Huff in this series emphasizes the need of more precise information regarding experimental animals also and Huff cites data supporting this view.

There are four principal methods by which changes in strains of pathogens might occur: (1) Assortment of biotypes from an originally composite strain—one comprising several biotypes or potential biotypes; (2) temporary, semipermanent or permanent change due to environment, including particular hosts; (3) mutation; (4) recombinations resulting from hybridization, including crossing between biotypes within a species.

That some of the supposed change in virulence of certain pathogenic organisms is not real change in virulence at all but merely apparent change due to population shifts within a strain comprising several biotypes is very clear from studies with certain plant-pathogenic fungi, notably smut fungi. A few years ago the ultimate in refinement of technic was supposed to have been attained when lines were derived from single spores. It is now known, however, that the ordinary smut spore (chlamydospore) has a diploid nucleus; hence, when the spore germinates and produces a promycelium that in turn produces four haploid sporidia, as is true of many smut fungi, all four may be different in observable characters, including sex and pathogenicity. Naturally, then, changes in pathogenicity may appear to have occurred in serial passages through several varieties of host plants. The apparent change, however, is due only to the selective effect of the host plants on two or more biotypes that differ in pathogenicity on the varieties of host plants. Similarly, when several varieties of wheat are inoculated with field collections of the wheat stem rust fungus, *Puccinia graminis tritici,* certain of the varieties may become heavily rusted while only a few pustules appear on others. When transfers are made from these few pustules to the apparently resistant varieties heavy infection may result, and it appears that the virulence of the rust has increased. It has been shown conclusively, however, that this is not true, but that the original rust comprised several races, each of which remains constant when isolated in pure form. Experience with such pathogenic fungi as the smuts and rusts in which the genetic status of single-spore lines can be determined makes it advisable to be cautious in interpreting changes in pathogenicity. Several years ago there appeared to be considerable evidence that decided changes in virulence might occur and that plant pathogens could adapt themselves easily to resistant or semi-resistant hosts. Subsequent investigations, however, have shown that this conclusion was erroneous, in most cases at least, as the apparent adaptation was due to the presence of several races in the original inoculum. Likewise, it was originally thought that saprophytic species of Actinomyces in the soil and on grass became pathogenic to animals after entering the body. Careful investigations, however, indicate that this is not true. That changes in virulence of pathogenic microorganisms occur is clear; but the magnitude and permanence of the change in a single pure line or biotype requires further investigation.

Studies by Watson presented in the second paper indicate change in the virus causing distemper of certain animals. Strains originally obtained from fox, mink, and dog appeared slightly different in infectivity and pathogenicity at first, but no differences were observable after serial passage in ferrets, for which they are very virulent, following which they appear to have lost much of their virulence for dogs and foxes, the original hosts. There is evidence from this and other investigations that animal viruses may adapt themselves to new hosts or to new environments in the same host (such as change from viscerotropic to neurotropic types in the virus of South African horse sickness and in that of yellow fever in man) as a result of intercranial passages in white mice, without resuming their original character when reintroduced into the original host or environment. If the original virus was pure in a genetic sense, the results must be interpreted as indicating that the environment caused an irreversible change, the production of a new strain. The difficulty or impossibility of assuring the "genetic purity" of a virus is, of course, obvious. Possibly further light on the probable nature of such changes in viruses is furnished by experiments on changes in tobacco-mosaic virus.

Kunkel has shown in his paper that when a large dose of virus is used for serial inoculations of tobacco plants, the disease remains the same, regardless of the number of transfers. If a minimal infective dose is used at each transfer, however, the tobacco mosaic virus will eventually be lost and a pathogenically different but closely related virus will be obtained. That some type of variation comparable with genetic variation accounts for the change appears probable from the following: Local lesions appear on leaves of *Nicotiana glutinosa* L. inoculated with a proper dilution of virus from a tobacco plant showing typical mosaic symptoms following inoculation with tobacco mosaic virus only. If healthy tobacco plants are then inoculated with virus obtained from the individual lesions on *N. glutinosa,* from 0.5 to 2 per cent of the plants develop a disease that is distinct from tobacco mosaic. Apparently, than, a small percentage of the virus particles are different from those causing typical tobacco mosaic and may be termed variants, 130 of which have been obtained. The evidence seems to indicate that the great majority of new virus particles produced in the host plants are like their "parents," but a few are different. The small population of variants appears to be overgrown and suppressed by the large population of the original virus unless methods such as the above are used to isolate them and permit their development free from the unequal competition with the vastly larger numbers of the original virus. This interpretation is supported by observations on fungi, where circumstantial evidence can be confirmed by direct evidence. The variants may again vary in the same manner as the original virus, but it is significant that the "parental type" can always be maintained and that the variants may be either more or less virulent than the "parents."

Although species of bacteria pathogenic to animals may remain stable through thousands of generations in the laboratory or in the animal body, most, if not all, sometimes undergo variation. The most commonly observed type of variation is the change from Smooth to Rough colony form, often with changes in virulence, antigenic composition, cell morphology, sensitivity to bacteriophage, and hydrophobe or hydrophile properties. The change from S to R occurs readily in many species, but reversion, or R to S, is rare. The variants often are very stable. As change in antigenic composition often occurs, the importance of this type of variation is evident. Perhaps one of the best known R variants is the so-called BCG, obtained by Calmette *et al.* from an old culture of tubercle bacilli and probably an R variant from the normal type. BCG is low in virulence and there is evidence that it lacks an antigenic component present in normally virulent tubercle bacilli; hence it appears to be unsuitable for immunizing purposes, as was originally claimed. Furthermore, some investigators have obtained normally virulent S variants from BCG and have then attenuated the variant to the level of BCG. Whether this means that genetic variation has occurred or that there has been merely temporary modification is not yet clear. However, the tentative explanation offered by Konst in his paper seems reasonable in the light of results obtained with fungi. He states "There is no direct evidence to explain these cycles, but the following suggestion seems worth making. The avirulent strain BCG is so unstable that it produces virulent Smooth types (Reed, Orr, and Rice 1934). These Smooth types became predominant when Watson injected the mixed cultures into guinea pigs and consequently were reisolated as the prevalent type. However, such Smooth types are known to be unstable, and during the prolonged incubation in culture they produced attenuated strains that gradually replaced the virulent type."

On the other hand, it is suggested from the work with pneumococci reported by Reed in his paper that variants from one type may be induced to assume the character of another type if grown in a menstruum containing the specific polysaccharide characteristic of the latter; and that the newly acquired character is passed on from generation to generation. Is this a directive influence of the environment or is it a selective influence on cultures comprising several biotypes? This question can be answered satisfactorily only by rigidly controlled investigations on the behavior of lines of bacteria derived from single organisms.

That bacterial cultures derived from single cells can be attenuated is shown by Riker's work with *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.*, the organism causing crown gall in plants (paper by Riker). A single-cell culture that had been constant in pathogenicity for 10 years became attenuated by 10 to 20 successive transfers in glycine and related compounds but were restored to full virulence by cultivation on routine yeast-infusion-sugar media. In this case, therefore, the change was reversible and in the nature of phenotypic variability rather than genetic variation. As Riker says, "The original purity of a bacterial culture is essential for studies on variations. In some cases the changes in virulence discussed appear gradually, indicating that cultures can be 'educated.' In others, these changes appear to be relatively sudden and complete. Both kinds of change suggest 'genetic' problems of scientific interest and basic importance which, when solved, will doubtless have valuable biological applications. Since pathogenicity must be defined in terms of the susceptibility of the host, a concept of variation in the disease-producing ability of bacteria must consider not only the genetic constitution but also the physiological condition and the environment of the host."

Protozoa parasitic to animals are reported to be modified considerably by environmental factors. Thus, there is evidence that trypanosomes may acquire antibody resistance, that they change in antigenic composition, and that the acquired characters may be transmitted through many asexual generations. Similarly, they are known to develop resistance to drugs; if treated with repeated subcurative doses, the parasites may become resistant to larger doses than are ordinarily required to cure. Free-living forms, such as Paramecium, also acquire resistance to various chemicals and may retain the newly acquired character through many asexual generations, eventually losing it if grown on media free of the specific chemical, and losing it promptly when sexual reproduction occurs (Paper No.7). Clearly, this ability to develop *Dauermodifikationen,* or persistent modifications, is very important in enabling the parasites to overcome the defensive mechanisms of the host and to survive, but the nature of the change is still unknown. It is well known that there may be many races or strains within species of protozoa. Furthermore, it seems significant that a large proportion of the population is killed when first brought into contact with immune sera or with poisons. This has led some investigators to postulate the existence of several biotypes or strains in the original population of parasites, with the elimination of all but the resistant ones. If this is true, however, why should the new characters be lost eventually? And if a gene change accounts for the new character, why should conjugation result in its loss? Various animals have developed defense mechanisms against invading parasites and non-infectious foreign materials; it is not surprising that animal parasites also should have this ability. The transmission of a temporary character to hundreds of generations of asexually produced offspring, however, is more difficult to understand, as is the loss of the character in sexual reproduction. As pointed out by Taliaferro and Huff in their paper, almost all physiologic characters of parasitic protozoa are more or less dependent on their environment, the living host; therefore, the genetic constitution of the host must be considered in studies on the genetics of the parasites. Possibly too much trust has been placed in what a clon is supposed to represent. As a clon is a population of organisms derived by aexual propagation from a single individual, the implication is that all of the individuals should be alike, barring mutations resulting from chromosomal aberrations or gene changes. But the reservation "barring mutations . . . " is sometimes of the greatest importance. For example, certain haploid, unisexual clons of some smut fungi derived from single cells may within a few months comprise hundreds of biotypes differing in virtually every recognizable character.

In any case, it is important to learn whether protozoa and other pathogenic organisms can readily change in such ways as to nullify control measures or whether different measures must be taken against genetically different races.

There is now very little evidence that plant pathogenic bacteria and fungi can adapt themselves to new hosts and new environments, unless the species comprises biotypes or produces new ones with the necessary genes. It is worthy of emphasis that 25 years ago the connotation of adaptation and "bridging hosts" was accepted as unquestioned fact. But the word species now has mostly symbolic meaning in plant pathology. A species is a group of biotypes that can be distinguished with reasonable facility and certainty, almost exclusively by morphologic characters, from other groups of biotypes. As far as now known, the fact that the species comprises many biotypes instead of one and that it is a varying concept rather than a fixed entity may have relatively little practical significance in the case of those diseases that are controlled by chemical treatment, such as seed or soil disinfection and spraying or dusting, although there are indications that biotypes within a species may in fact differ in their resistance to fungicides. The situation is much more serious, however, in connection with controlling plant diseases by growing resistant varieties.

Variability in the resistance of varieties of crop plants has been observed for many years. Varieties that appeared to be immune for a time lost their resistance in certain regions or in certain seasons. Various explanations were offered, one of which was that the pathogen adapted itself to the variety. Experiments were made to test the theory and the results apparently proved it to be correct. But these early investigators did not realize several basic facts: 1. A species of pathogenic fungus may comprise an indefinite number of morphologically indistinguishable races that differ profoundly in parasitism; 2. A variety of crop plant may consist of a morphologically homogenous but physiologically heterogeneous population. This is particularly true of cross pollinated plants such as corn and rye, but it is true also of some predominantly self pollinated plants, such as flax. The individual plants, then, may vary greatly in resistance to a single pure line of the pathogen because the plants themselves are genetically different with respect to disease resistance; 3. To have genetic status a culture must be derived from single spores or cells whose nuclear condition is known; 4. Mutation and hybridization account for almost incomprehensibly great variation in some fungi. Without repeating details, then, it can be stated categorically that many conclusions regarding adaptation of pathogenic fungi to new hosts were based on misinterpretations due to insufficient information regarding these basic facts.

The reaction of a pure line of a host plant to a pure line of a pathogen depends on the interaction of a complex set of factors: the genetic factors in the particular variety of host and their effects at different stages in its development; the genetic factors in the particular races of the pathogen; the effect of environmental factors, such as soil moisture, soil nutrients, light, temperature, etc., on the host and on the pathogen; the effect, in some cases, of biotic environment of very complex nature; and the effect of environment on the physiologic interaction between host and pathogen. The complexities of all possible combinations of interactions are extremely great, but they are real, not imagined. Great as the range of variability may sometimes be, however, the reaction of a given variety to a given biotype varies only within limits imposed by the genes, and the genes themselves remain unchanged except when mutation occurs. That is, temporary modifications are not inherited, although the effects of certain environments may persist for some time.

Mutation and hybridization are responsible for very extensive variation in many plant pathogenic fungi (papers by Craigie, Rodenhiser, and Christensen). Hybridization may occur between biotypes within a species, between varieties within a species, between species themselves, and even between different genera. In some groups of fungi sexual fusions are prerequisite to parasitism. In the smut fungi and rust fungi, for example, the pathogenic phase is either wholly or predominantly dicaryotic. Nuclei of opposite sex are associated in pairs during the period of growth and they finally fuse at the time resting spores are formed. Before infection again occurs, therefore, segregation and recombination occur. As there may be very many biotypes within the species, each differing in one or more characters, it is evident that this crossing between biotypes within a species may result in the production of a very large number of new biotypes. It is not uncommon in the smut fungi to find that among 40 or 50 haploid segregates from a cross no two are identical with each other or the parents. The characters of the different lines often intergrade so much as to form almost a continuous series; for example, the cultures of all segregates of a cross may be of the same general color, but when all the cultures are grouped on the basis of color they may constitute so closely an intergrading series that one gets the impression that all had been painted with the same paint and brush, with decreasing dose of paint as the brush was moved across the series. This is true of other characters also. The importance of this fact is this: If there are such almost imperceptible intergradations with respect to visually observable characters, this also may be true of pathogenicity and general physiologic characters that are harder to detect.

It is known that there are many degrees of pathogenicity and of sex among segregates from crosses, and many differences are too slight to be observable unless one is fortunate enough to find the appropriate criteria. This is well illustrated by some rust fungi. Uredial clons of *Puccinia graminis tritici* derived from crossing races or from selfing a heterozygous race may appear identical in pathogenicity on a dozen varieties. But their pathogenicity for a thirteenth variety may be entirely different; there often is a remarkable degree of specificity of pathogenicity for certain wheat varieties, some of which are virtually indistinguishable except by reaction to rust.

It is worthy of emphasis that even a clon, if heterozygous, may yield a dozen or more parasitic races, biotypes, when sexual reproduction occurs. This is shown clearly in the paper by Craigie. It should be emphasized also that extensive experiments in hybridizing smut fungi and rust fungi show that when biotypes differing in several characters are crossed the characters may be recombined in many different ways in the progeny. Furthermore, it is very clear that this process of hybridization, with consequent recombinations and segregation of new biotypes, is going on in nature.

The situation is complicated enough by this crossing of biotypes within a species, but still "wider" crosses also can be made and there is circumstantial evidence that they are being made by nature also. In the smut fungi crosses between species and between genera indicate that unusual biotypes or strains or races of pathogens can be produced as a result of wide crosses, just as unusual varieties of crop plants can be produced occasionally as a result of wide crosses. This is true also of intervarietal crosses in *P. graminis,* when crosses are made between varieties that differ so much in morphology and pathogenicity that it would be justifiable to consider them distinct species.

The rust and smut fungi are not unique among plant pathogenic fungi with respect to variation resulting from hybridization; they merely have been studied more extensively than most others.

Mutation is very common in many plant pathogenic fungi. It probably occurs in bacteria also, in the form of sector variants in cultures. But the evidence for mutation probably is clearest in some of the smut fungi, because haploid lines can be obtained from single, haploid cells (sporidia) that propagate vegetatively. It has been demonstrated that some such lines are relatively stable while others are extremely mutable. When crosses are made between stable and mutable lines, segregation for these characters takes place, showing that the tendency to mutate is due to genetic factors. Within a few months several hundred mutants may arise from a monosporidial line. They may differ slightly or greatly from their parents in virtually every observable character, including pathogenicity. Although most mutants appear to be less pathogenic than their parents, some are more pathogenic. The mutants may cross with each other and produce still other biotypes.

The number of biotypes that can be produced as a result of hybridization and mutation is illustrated well by experiments on *Ustilago zeae,* the common corn smut organism. From two haploid sporidia there were obtained by the writer and associates at least 5,000 biotypes within a period of 4 years.

Apparent mutation is extremely common in many fungi with multinucleate spores or with spores in which the nuclear condition is not known. It is possible that chance assortment of nuclei carrying different genes may account for the production of new biotypes in some fungi, as hyphal anastamoses may lead to heterocaryosis and subsequent reassortment of nuclei in hyphal branches and spores. But what seems particularly impressive is the tremendous number of biotypes that may arise by mutation in lines derived from single haploid cells.

In interpreting the changes in microorganisms, then, it is essential to know their genetic condition and that of their hosts. Phenotypic variability must be distinguished from genetic variation. Much remains to be learned, and it is clearly evident that it is important to make extensive and basic studies on the genetics of pathogens as a means of understanding the changes that occur and as a guide to practical procedures.

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